A RETROSPECTIVE STUDY OF DISEASE PROCESSES IN MANED WOLVES (CHRYSOCYON BRACHYURUS) IN NORTH AMERICAN ZOOLOGICAL INSTITUTIONS WITH EMPHASIS ON UROLITHIASIS, INFLAMMATORY BOWEL DISEASE, AND NEOPLASIA

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A RETROSPECTIVE STUDY OF DISEASE PROCESSES IN MANED WOLVES (*CHRYSOCYON BRACHYURUS*) IN NORTH AMERICAN ZOOLOGICAL INSTITUTIONS WITH EMPHASIS ON UROLITHIASIS, INFLAMMATORY BOWEL DISEASE, AND NEOPLASIA

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Abstract: The objective of this retrospective study is to summarize causes of disease and mortality in maned wolves (*Chrysocyon brachyurus*) in the North American Species Survival Plan Program (SSP) population. This information will inform and enhance animal health, husbandry, and conservation efforts. Pathology reports were requested from all zoological institutions housing maned wolves between 1930 and 2021. Data were reviewed and cause of death (COD) and reported diseases were summarized and compared by age group, organ system and disease process. One hundred and seventy-one wolves, 82 females and 89 males, met the inclusion criteria. The majority were geriatric (>11 yr; n = 96) or adult (2–11 yr; n = 67). Noninfectious diseases were the most common COD by process (n = 94; 54.9%). For COD by organ system, diseases of the digestive (n = 41) and urinary (n = 34) systems were most common. Neoplasia was the most common noninfectious COD and was the primary COD in 37 wolves (21.6% overall; 39.4% of noninfectious diseases). A total of 145 benign (n = 72) and malignant (n = 73) neoplasms were diagnosed in 44 individuals. Dysgerminoma was the most commonly reported tumor (n = 18), and was the most common neoplastic COD (n = 8). Cystinuria or urolithiasis (n = 71) and gastritis, enteritis, enterocolitis, or colitis (n = 50) (overall and grouped in each system due to presumed common underlying cause) were also common but were more often reported as comorbidities than as COD (n = 16 and n = 11, respectively). Infectious COD were reported in 17 wolves and included babesiosis (n = 4), acanthocephalans (n = 2), and one viral infection. Infections with a variety of bacteria in different organ systems were a COD in eight wolves.

INTRODUCTION

Known locally as lobo guará, aguará guazú, lobo de crin, and native to South America, the maned wolf (*Chrysocyon brachyurus*), is a rust colored, neotropical canid with a shaggy, dark mane.[29] Its range extends across central and eastern South America in northern Argentina, south and central Brazil, Paraguay, Bolivia, and southern Peru (Peruvian Pampas del Health).[14,29] The maned wolf is the fourth largest canid and is the largest canid in South America. It is the only member of its genus, and is more closely related to the bush dog (*Speothos venaticus*) than to wolves or foxes. The maned wolf is an omnivore with up to half of its diet consisting of plant material. It is listed as near threatened by the International Union for Conservation of Nature (IUCN)[20] and is considered regionally endangered in Argentina. The current free-ranging maned wolf population is estimated to include approximately 17,000 mature individuals, the majority of which are in Brazil. Threats include habitat destruction and deforestation due to agriculture, timber harvesting, ranching, infrastructure development, vehicle strike, hunting and human persecution, and native and introduced infectious diseases.[20]

Maned wolves have been managed in North American (NA) zoos since the 1930s (majority after 1960), and in 1985 the Association of Zoos and Aquariums (AZA) created the Maned Wolf Species Survival Plan (SSP) Program.[34] Its goals are to protect and sustain maned wolves through *ex situ* breeding, health and education programs, and to support *in situ* research and conservation

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projects.[34] Outcomes of this work include identification of health problems in managed and free-ranging animals. Recognized noninfectious diseases in the NA zoo population include idiopathic inflammatory bowel disease (IBD), cystinuria and urolithiasis, gastric dilation/volvulus, and ovarian dysgerminoma.[6,7,12,19,25] Reported infectious disease processes include canine distemper virus, parvoviruses, and babesiosis.[22,23,27,37] Most of the existing literature related to maned wolf diseases is in the form of case reports or case series focused on specific problems in the species. The goal of this retrospective review is to expand our understanding of health, disease, and causes of mortality in the NA maned wolf population.

MATERIALS AND METHODS

A search of the Zoological Information Management System (ZIMS) database, archived SSP information, and institutional historical records was performed to create a list of all maned wolves that have been managed in NA zoos. Pathology reports were subsequently requested from all institutions that managed wolves between 01 January 1930 and 31 December 2021. Data from these reports were reviewed and inclusion criteria for this study required that all of the following be included in the pathology report: historical medical information, gross postmortem and histological examination descriptions and diagnoses, and final comments and interpretations.

Data were categorized by sex (male, female, undetermined), age group (neonate: $<$ 30 days; puppy: 30 days to 6 months; juvenile: 6 months to 2 years; adult: 2 to 11 years; geriatric: $>$ 11 years), and cause of death (COD). COD determinations were based on the conclusions in the final comment from the referral pathologist, which took into consideration all of the case materials for an individual animal (majority of cases). Comorbidities were disease processes that were present at the time of death but not considered the COD. These may have been associated with, sequela to, or independent of the COD. COD and comorbidity information was then categorized by the authors of this report. In cases in which a clear conclusion/COD was not provided by the referral pathologist, the authors of this report reviewed all of the available case materials and determined and categorized the COD and comorbidities based on all available case information (clinical history, necropsy, histology, ancillary diagnostics). COD was further categorized by body system and disease process. Body system categories were cardiovascular, digestive (including liver), endocrine, hematolymphatic, integumentary, musculoskeletal, nervous, reproductive, respiratory, special senses, and urinary. Also included as body system categories were body cavity (e.g. pleural, peritoneal) and a generalized category in which a single disease process was reported in multiple organ systems. All gross and histologic diagnoses with a severity of mild, moderate, severe, or marked were compiled and included in data analysis. For cases where multiple disease processes were seen, the COD was the disease process that was likely to have been most clinically significant based on case review and referral pathologist diagnosis/es and comments coupled with the interpretation of the authors. Diagnoses not considered the primary COD were categorized as comorbidities. For animals that were euthanized, the primary disease process leading to euthanasia was recorded as the COD. Disease process categories were noninfectious, infectious, inflammatory (no specific etiology [NSE]), and undetermined. Animals with an infectious COD were those in which antemortem diagnostics, postmortem diagnostics, and/or histology confirmed an infectious organism as the cause of disease and death; inflammation may have been present or absent. Inflammatory (no specific etiology [NSE] were those COD with inflammation but no identified underlying cause. For example, enteritis or pyelonephritis in the absence of an identified infectious disease process for either (e.g., bacterial infection) or urolithiasis for the latter would be included in this category. Noninfectious COD were those in which a disease process that was neither infectious nor inflammatory (NSE) was identified (e.g., urolithiasis). Undetermined COD were those in which none of the conditions of the other categories was identified.

Descriptive statistics were used to summarize COD by body system and disease process. Linear regression analysis was performed using R statistics (lme4 package) to determine if there were any trends in gastrointestinal system inflammation over time. Significance was set at $P < 0.05$.

RESULTS

Information from 639 individual maned wolves was available in the ZIMS, institutional and the SSP databases. This included 158 geriatric animals (75.83.0 = male, female, undetermined, respectively), 204 adults (104.100.0), 24 juveniles (12.12.0), 20 puppies (12.8.0), and 233 neonates (43.41.149). The first available animal record was from a maned wolf that was received into a zoological collection in 1933. The majority of records were from animals received into or born at zoos

Table 1. Cause of death by disease process and age group.

Disease category	Neonate	Puppy	Juvenile	Adult	Geriatric	Total
Noninfectious	2	0	3	34	55	94
Infectious	0	1	2	10	4	17
Inflammatory-NSE[a]	0	0	0	14	22	36
Undetermined	0	0	0	9	15	24
Total	2	1	5	67	96	171

[a] NSE, no specific etiology.

after 1960 (n = 634). Twenty animals (10.10.0) were reported to have been wild caught and 16 (10.6.0) had a birth type listed as undetermined. All wild caught wolves entered zoo collections prior to 1990.

Partial to complete pathology reports from 414 individuals (153.154.107) from 37 zoos were received and available for review. Of these, 171 (89.82.0) met the study inclusion criteria (26.8% of all animals; 41.3% of animals with a pathology report). Ages ranged from 10 to 6,316 days (17 years 3 months 6 days). The majority of individuals that met the study inclusion criteria were geriatric or adult (n = 96 and n = 67, respectively; 95.5% overall). The remainder were juveniles (n = 5), puppies (n = 1), or neonates (n = 2). All but two (both females; geriatric at the time of death) were captive born (Supplemental Table 1).

Among general disease categories, noninfectious diseases were most common and the COD in over half of the study population wolves (n = 94; 54.9%; Table 1). They were also the most common COD regardless of age group, except in puppies. Noninfectious diseases were reported as a COD more than twice as often as inflammatory diseases with no specific etiology (NSE) (n = 36; 21.1%) and more than five times as often as infectious diseases (n = 17; 9.9%). A COD was undetermined in 24 (14%) wolves.

The most common COD by body system were those of the digestive (n = 41; 23.9%) and urinary systems (n = 34; 19.9%). All of the former and all but one of the latter were in geriatric and adult wolves (Table 2). Musculoskeletal or cardiovascular system disease COD were relatively common in geriatric wolves (n = 14; 8.2% and n = 12; 7.0%, respectively). Diseases of the reproductive (n = 11; 6.4%), nervous (n = 11; 6.4%), hematolymphatic (n = 9; 5.3%), integument (n = 9; 5.3%) and respiratory (n = 4; 2.3%) systems were less commonly reported as COD. Generalized diseases, a single disease process that affected multiple organ systems, were the COD in 11 wolves (6.4%). Diseases of the endocrine system, that originated in a body cavity, or for which the site of origin was undetermined (n = 1; 0.06% for each) were relatively uncommon.

The most common noninfectious COD was neoplasia (n = 37; 21.6%; Tables 3 and 4). All but one neoplastic COD were in geriatric and adult wolves. Neoplasia as a COD was three times more common in geriatrics than adults. As a noninfectious COD, it was more than twice as common as metabolic disease (n = 16; 9.4%). A wide variety of tumors, both benign (n = 72) and malignant (n = 73), were reported in 44 individuals (Supplemental Table 2). Some individuals had multiple different benign and/or malignant tumor types. Ovarian dysgerminoma was the most commonly reported tumor (n = 18) and alone or with related complications (e.g. rupture with hemoabdomen), was the most common neoplastic COD (n = 8). Astrocytoma was the only neoplastic cause of death in a juvenile wolf. Neoplasia was not reported as a COD in neonates.

Urolithiasis was reported in 71 wolves (49.22.0; 41.5% of study animals; Supplemental Figure 1) and was the COD in almost a quarter of affected animals (n = 16; 9 adults and seven geriatrics; 9.4% overall; 22.6% of wolves with urolithiasis; Table 3). It was the only metabolic COD. The only reported stone type was cystine. Related complications included bladder rupture (n = 3), hydronephrosis, nephritis, urethritis, and ascending bacterial infection with sepsis.

Other relatively common noninfectious COD included degenerative processes (n = 12) and trauma (n = 12). The former was most often due to spondylosis. It was reported in 20 geriatric and adult wolves and was reported as a COD (reason for euthanasia) in six geriatric wolves. Trauma was seen in all age groups except puppies and included accidental self and conspecific trauma. An additional notable noninfectious COD was gastric dilation and volvulus (n = 7).

Inflammation of undetermined cause (NSE) as a COD most often affected the digestive (n = 21; 12.3%) or urinary (n = 10; 5.8%) systems (Table 5). In the digestive system, enteritis, colitis, or gastritis was diagnosed overall in 50, 15 and 27 wolves, respectively, and overall was the COD

Table 2. Cause of death by body system.

Organ System	Neonate	Puppy	Juvenile	Adult	Geriatric	Total
Digestive	0	0	0	21	20	41[d]
Urinary	0	0	1	14	19	34[e]
Musculoskeletal	2	0	1	4	14	21[f]
Cardiovascular	0	0	1	4	12	17[g]
Generalized[a]	0	0	1	3	7	11[h]
Reproductive	0	0	0	2	9	11[i]
Nervous	0	0	1	8	2	11[j]
Hematolymphatic	0	1	0	4	4	9[k]
Integument	0	0	0	5	4	9[l]
Respiratory	0	0	0	2	2	4[m]
Endocrine	0	0	0	0	1	1[n]
Body Cavity[b]	0	0	0	0	1	1[o]
Undetermined[c]	0	0	0	0	1	1[p]
Total	2	1	5	67	96	171

[a] Generalized = single disease process in multiple organs.
[b] Body cavity = undetermined source for cavitary process.
[c] Undetermined = undetermined source for process.
[d] Digestive, inflammatory-NSE (n = 21): gastritis, enteritis, enterocolitis and/or colitis = 11, enteric and mesenteric lymphangiectasia = 1, gastric ulcer = 2 (both with peritonitis, one with perforation), cirrhosis = 5, pancreatitis = 2; noninfectious (n = 14): gastric dilation/volvulus = 7, neoplasia = 4 (hepatocellular carcinoma [n = 2], esophageal carcinoma [n = 1], pancreatic ductal adenocarcinoma [n = 1]), dehiscence of biopsy site with septic peritonitis = 1, ruptured hepatic cyst with hemoabdomen = 1; noninfectious-NSE = 1: intestinal rupture =1; infectious (n = 6): acanthocephalan = 2, bacterial = 3, viral = 1 (suspected adenovirus 2); Undetermined (n = 0).
[e] Urinary, inflammatory-NSE (n = 10): chronic nephritis, glomerulonephritis, or pyelonephritis = 10; noninfectious (n = 24): urolithiasis = 16 (2 with bladder rupture), neoplasia = 5 (renal carcinoma [n = 2]; transitional cell carcinoma [n = 2]; bladder Teratoma [n = 1]), bladder rupture not related to urolithiasis = 1, medullary fibrosis = 1, nephrosclerosis = 1; infectious (n = 0); undetermined (n = 0).
[f] Musculoskeletal, inflammatory-NSE (n = 0); noninfectious (n = 21): trauma = 9 (1 iatrogenic), spondylosis = 6, chronic osteoarthritis =2, neoplasia = 3 (sarcoma [n = 3]), congenital abnormality/open fontanelle = 1; infectious (n=0); undetermined (n=0).
[g] Cardiovascular, inflammatory-NSE (n = 1): systemic vasculitis; noninfectious (n = 3): neoplasia = 1 (lymphoma), shock secondary to capture myopathy (n = 1), arterial sclerosis with thrombi and infarcts (n = 1); infectious (n = 3): bacterial = 2, dirofilariasis = 1; undetermined (n = 10).
[h] Generalized, inflammatory-NSE (n = 0); noninfectious (n = 1): cachexia; Infectious (n = 1): bacterial tendonitis, dermatitis and sepsis; undetermined (n = 9).
[i] Reproductive, inflammatory-NSE (n = 0); noninfectious (n = 11): neoplasia = 9 (dysgerminoma [n = 8], ovarian leiomyosarcoma [n = 1]), reproduction complications = 2 (in utero fetal death with metritis = 1, cystic endometrial hyperplasia = 1); infectious (n = 0); undetermined (n = 0).
[j] Nervous, inflammatory-NSE (n = 2): encephalitis = 1, meningoencephalitis = 1; noninfectious (n = 7): neoplasia = 4 (neurofibrosaroma [n = 1], astrocytoma [n = 1], aortic body carcinoma [n = 1], undifferentiated anaplastic carcinoma brain [n =1]), degeneration/progressive motor neuron disease = 1, trauma = 1; noninfectious-NSE = 1 (meningeal cholesteatoma); infectious (n = 0), undetermined (n = 2): paresis =1, idiopathic epilepsy = 1
[k] Hematolymphatic, inflammatory-NSE (n = 0); noninfectious (n = 3): neoplasia = 3 (lymphoma [n = 2], malignant fibrous histiocytoma [n = 1]); infectious (n = 4): babesiosis (n = 4); undetermined (n = 2): hemolytic disease = 2.
[l] Integument, inflammatory-NSE.(n = 1): multifocal cellulitis (aseptic); noninfectious (n = 5): neoplasia = 4 (spindle cell sarcoma [n = 1], basal cell carcinoma [n = 1], mammary gland adenocarcinoma [n = 1], fibrosarcoma [n = 1]), trauma = 1; infectious (n = 3): bacterial = 2, siphonapteriasis (flea infestation) = 1; undetermined (n = 0).
[m] Respiratory, inflammatory-NSE (n = 2): interstitial pneumonia = 1, bronchopneumonia = 1; noninfectious (n = 2): trauma = 1, pulmonary metastatic neoplasia of undetermined origin = 1; infectious (n = 0); undetermined (n = 1).
[n] Endocrine, inflammatory-NSE (n = 0); noninfectious (n = 1): malignant pheochromocytoma = 1; infectious (n = 0); undetermined (n = 0).
[o] Body cavity, inflammatory-NSE (n = 0); noninfectious (n = 1): abdominal sarcoma = 1; infectious (n = 0), undetermined (n = 0).
[p] Undetermined, inflammatory-NSE (n = 0); noninfectious (n = 1): poorly differentiated adenocarcoma (near thymus) of undetermined origin = 1; infectious (n = 0); undetermined (n = 0).

in 11 (6.4%). Inflammation was generally lymphoplasmacytic (n = 27), eosinophilic (n = 19) or a combination of the two. Other COD in this category included perforating gastric ulcers in two wolves, pancreatitis in two, and enteric and mesenteric lymphangiectasia in one. Regression analysis revealed a positive relationship ($P = 0.0201$, $R^2 = 0.179$) between the numbers of enteritis cases over time (Supplemental Figures 2 and 3). An additional digestive system diagnosis was cirrhosis, chronic end stage hepatic disease, which was the COD in five wolves. In the kidney, glomerulonephritis (n = 5) or nephritis (n = 3) were most common. Inflammation was often lymphoplasmacytic

Table 3. Noninfectious causes of death by general category and age group.

Disease category	Neonate	Puppy	Juvenile	Adult	Geriatric	Total
Neoplastic	0	0	1	9	27	37[a]
Metabolic	0	0	0	9	7	16[b]
Trauma	1	0	2	5	4	12[c]
Degenerative	0	0	0	2	10	12[d]
Abnormal position	0	0	0	5	2	7[e]
Iatrogenic	0	0	0	2	1	3[f]
Reproductive complications	0	0	0	1	1	2[g]
Congenital	1	0	0	0	0	1[h]
Noninfectious-NSE	0	0	0	1	3	4[i]
Total	2	0	3	34	55	94

[a] Neoplastic, ovarian dysgerminoma (n = 8), musculoskeletal sarcoma (n = 3), hepatocellular carcinoma (n = 2), renal carcinoma (n = 2), urinary bladder transitional cell carcinoma (n = 2), disseminated lymphosarcoma/lymphoma (n = 2), n = 1 for each of the following: abdominal sarcoma, aortic body carcinoma, cardiovascular lymphoma, esophageal carcinoma, pancreatic duct adenocarcinoma, malignant pheochromocytoma, malignant fibrous histiocytoma, poorly differentiated adenocarcinoma of undetermined origin, undifferentiated anaplastic carcinoma (brain, lung), metastatic squamous cell carcinoma of undetermined origin in the lung, astrocytoma, neurofibrosarcoma, urinary bladder teratoma, ovarian leiomyosarcoma, mammary gland adenocarcinoma and integumentary: fibrosarcoma, spindle cell sarcoma, and basal cell carcinoma.

[b] Metabolic, urolithiasis (n = 16; 3 with bladder rupture).

[c] Trauma, leg fracture (n = 5), n = 1 for each of the following: maternal trauma, vertebral column fracture, toe amputation, self-induced skin-trauma, bladder rupture without urolithiasis, head and thoracic trauma, spinal cord hemorrhage and myelomalacia (suspect intervertebral disc disease).

[d] Degenerative: spondylosis (n = 6), chronic arthritis (n = 2), n = 1 for each of the following: central and peripheral neuronal degeneration/progressive motor neuron disease, hepatic cyst rupture, chronic renal fibrosis, and tubular atrophy, nephrosclerosis.

[e] Abnormal position, gastric dilation and volvulus (n = 7).

[f] Iatrogenic, n = 1 for each of the following: intestinal dehiscence (biopsy site) with sepsis, complications related to leg fracture fixation (darting accident), shock (presumed secondary to acute rhabdomyolysis or idiosyncratic drug reaction).

[g] Reproductive complications, n = 1 for each of the following: in utero fetal death with metritis and sepsis, cystic endometrial hyperplasia with pyometra.

[h] Congenital, n = 1: open fontanelle.

[i] Noninfectious-NSE, n = 1 for each of the following: intestinal rupture, meningeal cholesteatoma, cachexia, arteriosclerosis with multifocal thrombi, infarcts. and necrosis.

and in some cases was associated with membranous or membranoproliferative glomerulopathy, glomerular synechia, tubular dilation (occasionally cystic), proteinosis, and/or interstitial fibrosis. It was associated with uremic stomatitis, gastritis and/or pneumonitis in two wolves and with anemia and secondary parathyroid gland hyperplasia in one.

Infectious diseases were a COD in 17 (9.9%) wolves. Bacterial (n = 8) and parasitic infectious (n = 8) were more often a COD than viral infections (n = 1) (Supplemental Table 3). Babesiosis (n = 4), enteric acanthocephalans (n = 2) and single reports of siphonapteriasis (flea infestation) with anemia, dirofilariasis, and hepatic adenovirus-2 infections were among the reported infectious COD.

The most common diagnoses overall in the study population were urolithiasis (n = 71), enteritis/enterocolitis (n = 50) and nephritis (n = 40) (Supplemental Table 4). Other findings with a severity of mild, moderate, severe, or marked reported in 20 to 30 individuals included: endocardiosis, gastritis, adrenal cortical hyperplasia, myocardial fibrosis, glomerulonephritis, degenerative joint disease/arthritis, and spondylosis. Findings in 10 to 19 individuals included: arteriosclerosis, ovarian dysgerminoma, hepatic lipidosis, pheochromocytoma, colitis, heart disease (NSE), interstitial (Leydig) cell tumor, cystitis, dermatitis, hepatitis, cataract, and cystic endometrial hyperplasia. Over 600 other diagnoses, each in nine or fewer individuals were also reported (Supplemental Table 4). Notable diagnoses among them included gastric dilation and volvulus (n = 7), nonsuppurative encephalitis (NSE) (n = 6), dilated cardiomyopathy (n = 4), cryptorchidism (n = 3), renal nematodiasis (*Dioctophyma renale*), and dirofilariasis (heartworm) (n = 2 each) and one of each of the following: corneal dystrophy, idiopathic epilepsy, and hydrocephalus.

DISCUSSION

Results of this comprehensive review of diseases and mortality information from maned wolves in North American zoos aligned with our understanding of several commonly recognized disease processes in this species. It also documents a wider

Table 4. Neoplasia as the cause of death by body system and age group.

Age group	Juvenile	Adult	Geriatric	Total
Reproductive	0	1	8	9[a]
Urinary	0	2	3	5[b]
Digestive	0	1	3	4[c]
Integumentary	0	2	2	4[d]
Nervous	1	2	1	4[e]
Musculoskeletal	0	1	2	3[f]
Hematolymphatic	0	0	3	3[g]
Cardiovascular	0	0	1	1[h]
Body Cavity	0	0	1	1[i]
Endocrine	0	0	1	1[j]
Respiratory	0	0	1	1[k]
Undetermined tissue origin	0	0	1	1[l]
Total	1	9	27	37

[a] Reproductive, dysgerminoma (n = 8), ovarian leiomyosarcoma (n =1).
[b] Urinary, renal carcinoma (n = 2), transitional cell carcinoma (n = 2), malignant cystic teratoma (n =1).
[c] Digestive, hepatocellular carcinoma (n = 2), pancreatic ductal adenocarcinoma (n = 1), esophageal carcinoma (n = 1).
[d] Integumentary, mammary gland adenocarcinoma (n = 1), basal cell carcinoma (n = 1), spindle cell sarcoma (n = 1), fibrosarcoma (n = 1).
[e] Nervous, aortic body carcinoma (n = 1), undiffereintiated anaplastic carcinoma of undetermined origin in the brain (n = 1), astrocytoma (n = 1), neurofibrosarcoma (n = 1).
[f] Musculoskeletal, poorly differentiated sarcoma (n = 1; presumptive liposarcoma), sarcoma (n = 1; scapulohumeral joint), sarcoma (n = 1; leg).
[g] Hematolymphatic, disseminated lymphosarcoma/lymphoma (n = 2), malignant fibrous histiocytoma/histiocytic sarcoma (n = 1).
[h] Cardiovascular, cardiac lymphosarcoma/lymphoma (n = 1).
[i] Body cavity, abdominal sarcoma (n = 1).
[j] Endocrine, pheochromocytoma (n = 1).
[k] Respiratory, metastatic squamous cell carcinoma of undetermined origin in the lung (n = 1).
[l] Undetermined tissue origin, poorly differentiated adenocarcinoma of undetermined origin near thymus (n = 1).

range of diseases than have previously been reported, reveals several causes of illness and death that may be underappreciated, and provides an opportunity to review and improve upon the ways in which we collect and evaluate morbidity and mortality-related information.

As has previously been reported, urolithiasis, gastrointestinal tract inflammation, and dysgerminoma were common in wolves in the current study.[6,7,12,25] When present, dysgerminoma and/or related complications more often resulted in death (8 of 18; 44.4%) than urolithiasis (16 of 71; 22.5%) or gastrointestinal tract inflammation (11 of 92; 11.9%). As comorbidities, each, especially gastrointestinal tract disease, can contribute to a decline in general health or potentially pre-dispose to other (e.g. opportunistic) disease processes.

Urolithiasis has been reported in both captive and wild maned wolves, and while a known cause of morbidity and mortality in the former, outcomes in the wild are unclear.[29,34] The most commonly reported uroliths in the species are composed of cystine, however combinations of calcium, phosphate, magnesium, potassium and/or struvite have also been reported.[6,7,12] Cystine was the only reported urolith in the current study, though it was not clear if identification was always based on composition analysis or making assumptions when uroliths or crystalluria were detected. Urolith compositional analysis is recommended whenever possible to ensure accurate identification and to inform interventions focused on mitigating their development.

In the domestic dog, cystine urolithiasis has been reported in a number of breeds and, interestingly, differences in breed predisposition appear to vary geographically. For example, commonly affected breeds in the United States include English bulldogs, Newfoundlands, and dachshunds, those in Germany include bulldogs and Chihuahuas, and terrier breeds are most commonly affected in the United Kingdom.[18] As an inherited metabolic disease, excessive urinary excretion of cystine, lysine, ornithine, arginine or other amino acids have been suggested as precursors in the development of urolithiasis in domestic dogs, cats, and humans.[4,6] As has already been mentioned, cystine urolithiasis has been a recognized husbandry and health challenge for maned wolves in managed settings for many years.[4,5,7,12,26,34] Genetic and nutritional factors have been suggested as predisposing factors in maned wolves, and one study suggested that a variable defect for cystine and dibasic amino acid reabsorption may be a factor.[6] This same study argued that nutrition was unlikely to be a factor since both wild and zoo animals with varying diets can both develop cystine urolithiasis. However, neither factor has been rigorously studied in this species.

In the wild, maned wolves are omnivores with a diet that varies seasonally and consists primarily of plant materials (~50%), small mammals, birds, and insects.[13,24] Maned wolves in zoos were initially fed high protein diets until it was suggested that they might predispose the wolves to urolithiasis.[3] High protein diets increase urine acidity, which increases the insolubility of cystine and can result in the development of renal and urinary bladder uroliths.[5,7,12,34] In response to clinical illness in maned wolves in North American zoos, numerous efforts to develop a diet that would reduce urinary cystine and urolith development began in 1990. Promising results were initially

Table 5. Inflammatory (NSE)* causes of death by body system and age group.

Organ system	Neonate	Pup	Juvenile	Adult	Geriatric	Total
Digestive	0	0	0	9	12	21[a]
Urinary	0	0	0	3	7	10[b]
Nervous	0	0	0	2	0	2[c]
Integument	0	0	0	0	1	1[d]
Respiratory	0	0	0	0	1	1[e]
Cardiovascular	0	0	0	0	1	1[f]
Total	0	0	0	14	22	36

[a] NSE, no specific etiology.
[b] Digestive, gastritis, enteritis and/or colitis (n = 11), cirrhosis (n = 5), pancreatitis (n = 2), gastric ulcer with peritonitis (n = 2; 1 with perforation), enteric and mesenteric lymphangiectasia (n = 1).
[c] Urinary, glomerulonephritis and/or nephritis (n = 8), pyelonephritis (n = 1), renal atrophy with tubular necrosis and nephrosclerosis (n = 1).
[d] Nervous, nonsuppurative encephalitis with perivascular cuffs and intracytoplasmic and intranuclear eosinophilic inclusions (n = 1; canine distemper virus suspected but not confirmed), meningoencephalitis with perivascular cuffs (n = 1).
[e] Integument, cellulitis with nonbacterial sepsis (n = 1).
[f] Respiratory, interstitial pneumonia (n = 1).
[g] Cardiovascular, systemic lymphohistiocytic (n = 1).

achieved. However, reports of a possible link with decreased reproductive success in 2002 raised concerns about possible diet-related complications, so many zoos subsequently returned to feeding commercially available, chow-based diets supplemented with a variety of fruits, vegetables, and small amounts of whole prey.[5,7,34] A follow-up study in 2006 confirmed a wide variation in maned wolf diets across North American zoos.[28,34] Neither the diet trials nor these differences in diet appear to have reduced the incidence of urolithiasis in maned wolves over the years (Supplemental Figure 1). Comprehensive studies to understand the cause/s for cystinuria in maned wolves and development of diets to reduce its incidence have not been performed in over a decade but are needed to develop strategies that reduce urolithiasis and improve overall animal health.[28]

In addition to urolithiasis, inflammatory bowel disease (IBD) is a recognized cause of clinical illness in maned wolves.[17,29] IBD describes a group of disorders that cause chronic inflammation in the gastrointestinal tract. In general, its cause is unknown although some suggest genetics, mucosal immunity and the intestinal microflora as important factors in its development in domestic dogs and cats.[21,33] In the current study, inflammation in the absence of an identified cause (NSE) was observed most commonly in the digestive system, and enteritis was the most common diagnosis. It was reported in almost 30% (n = 50) of maned wolves in the study; no sex predilection was seen. Interestingly, it was more often a comorbidity than the COD. However, morbidity related to enteric inflammation was poorly defined through this study and it could have important clinical effects (e.g. vomiting, diarrhea, loss of condition) that predispose to or complicate other disease processes. It was most often lymphoplasmacytic, eosinophilic or a mix of both, which is similar to IBD reported in captive red wolves.[31] Though less often reported, gastric dilation and volvulus (GDV) with or without splenic torsion was more often a direct COD than gastrointestinal inflammation. GDV, which causes vascular occlusion and acute hemodynamic collapse, is a medical emergency and immediate aggressive medical and surgical intervention contribute to but are not always a guarantee of a successful outcome.[19] It was the COD in all seven of the maned wolves in which it was reported. To date it has not been associated with a specific cause. An additional notable digestive system COD was cirrhosis (end-stage hepatitis; n = 5). It has not previously been reported in maned wolves. Underlying causes were not identified in any wolf in which it was diagnosed.

Enteritis was consistently diagnosed over the years of this study and appeared to be associated with a slow upward trend (Supplemental Figures 2 and 3). More cases were reported between 2009 and 2021 (n = 33) than were reported in the previous 25 years (n = 17). However, it is unclear if this represents a true increase in disease prevalence or if recognition of clinical disease has more recently biased clinical examinations and histologic review. The latter is hard to assess since standardized criteria for histologic evaluation of gastrointestinal tract inflammation, which is used in domestic dogs,[9,33,36] has not been developed for maned wolves. This highlights the need to adopt or modify existing diagnostic methods in order to allow consistent review

among pathologists. This will both aid in accurately differentiating normal background cell populations from disease and allow monitoring of disease progression or remission in response to diet or other management interventions. From a management perspective, diet modifications, some related to a 2009 diet survey performed by members of the AZA Nutrition Advisory Group, were proposed and adopted over the years.[28] However, to date, these modifications do not appear to have resulted in a decrease in inflammatory gastrointestinal tract disease. Interest in developing a safe, effective diet to reduce IBD-related morbidity and mortality in addition to reducing cystinuria remain high priorities for managers of maned wolves.

Interesting among the aggregated data were the number and types of tumors that have been diagnosed in maned wolves. One hundred forty-five benign or malignant tumors were reported in 44 individuals (Supplemental Table 2). This included a wide variety of tumor types in a number of different organ systems. Additionally, multiple benign, malignant or both tumor types were present in many individuals. Ovarian dysgerminoma was the most common tumor overall and was the most common neoplastic COD. Dysgerminomas are primordial germ cell tumors that arise in the ovary. They can grow quite large before being diagnosed. Complications such as metastatic disease or rupture with hemoabdomen can cause death. With the exception of maned wolves, this is a relatively uncommon tumor in domestic and nondomestic canids.[1,25] To date, a cause for this putative predisposition in maned wolves has not been identified.

Surprisingly, pheochromocytoma was the second most common tumor (benign = 10; malignant = 7) although it was only reported as a COD in one wolf. These neuroendocrine tumors arise from chromaffin cells in the adrenal medulla. They are generally uncommon in most species but are reported with some frequency in domestic dogs, cattle, horses, New World primates, and clouded leopards.[8,30] Clinical signs can be associated with tumor-related catecholamine excess that can produce hypertension, tachyarrhythmias, weakness/collapse, abdominal pain or vomiting. These symptoms are nonspecific and some can overlap with signs of IBD or other disease processes. Clinical evaluation to include diagnostics for pheochromocytoma (e.g. catecholamine excess, CT scan, cytology, biopsy, histology, immunohistochemical labeling) may be warranted in wolves that display these clinical signs to ensure they are not misinterpreted and to allow timely medical interventions.[8,32] Also and interestingly, approximately 75% of the pheochromocytomas were in males. This differs from findings in domestic dogs in which no sex predisposition has been identified.[32]

Although often a focus of case reports in the literature, infectious disease was not a common COD in the current study. Among infections were several case clusters. These include babesiosis and intestinal acanthocephalan infections. The former has been diagnosed in captive maned wolves in the midwestern United States and South America.[27] It is a tick-born protozoal disease. Infection targets red-blood cells and causes direct cell lysis and antibody-mediated erythrocyte destruction. Diagnosis is based on clinical signs (e.g., anemia, icterus), identification of characteristic intraerythrocytic parasites on blood films, histology, or PCR. Previous reports have increased awareness of the disease in maned wolves, and mitigation through tick management can decrease its occurrence. Acanthocephalans (syn. thorny-headed worms, spiny-headed worms) have been identified in maned wolves in the wild and in zoos.[2,10,16] In the United States, cases have been reported in Texas and were the result of infection with *Pachysentis canicola*, which has been reported in other carnivores in North and South America including striped skunks (*Mephitis mephitis)* in Texas.[2] Diagnosis can be challenging as these parasites may be shed sporadically and result in false negative fecal smear and/or float tests, and clinical signs can overlap with those of IBD. Death may occur secondary to transmigration of parasites through the intestinal wall with subsequent perforation and septic peritonitis. Despite maned wolves' known susceptibility to certain viruses including canine distemper virus, canine adenovirus and parvoviruses, viral infections were not commonly reported in our study.[11,15,35]

Neonates were under-represented in the current study. More than 200 individuals were listed in historical databases but only two met the study inclusion criteria. The reason for exclusion of the majority of these animals was because a complete pathology report was not available for review; histology was not performed in most cases. Historical information and/or gross necropsy information from many included descriptions of parental trauma, or in some cases, animals were listed as having disappeared and were assumed to have been consumed. Based on the historical information, parental trauma, which has been described as a common cause of neonatal mortality in captive maned wolves in

South America, was presumed to be a common cause of death in many of the neonates in this study.[22] For future cases, histology and testing for other disease processes, even if only performed on limited tissue sets, would improve our understanding of disease presence or absence in this group.

CONCLUSIONS

As in previous reports, urolithiasis and gastrointestinal tract inflammation (IBD) were commonly reported in this study and remain a challenge in the management of maned wolves in North American zoos. This highlights an ongoing need to understand factors that drive the development of these diseases and the importance of developing standardized methods for assessing disease and response to husbandry and health interventions. Doing so will inform changes that decrease morbidity and improve overall maned wolf health. Previously not documented but as in domestic canids, maned wolves develop many different types of benign and malignant tumors. They appear to be predisposed to developing dysgerminomas though to date, underlying mechanisms that drive their development have not been investigated or identified. Infectious disease is not a common cause of death in maned wolves in North American zoos, though regional variations exist. This and the fact that most maned wolves in North American zoos live into and beyond adulthood are a reflection of improvements and ongoing efforts to constantly adjust husbandry and health practices to care for this iconic species.

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Supplemental Table 1. Age and sex distribution.

Supplemental Table 2. Benign and malignant tumors by body system.

Supplemental Table 3. Infectious causes of death by disease type and age group.

Supplemental Table 4. Diagnoses with a severity of mild, moderate, severe or marked in the 171 maned wolves that met the study inclusion criteria. The totals are the number of individuals with a specific diagnosis.

Supplemental Figure 1. Urolithiasis in maned wolves by year. Individuals with a diagnosis of urolithiasis = 71; individuals in which urolithiasis was the COD = 16.

Supplemental Figure 2. Enteritis in maned wolves by year. Individuals with a diagnosis of enteritis = 50; individuals in which enteritis was the COD = 11.

Supplemental Figure 3. Enteritis diagnoses between 1983 and 2021. Linear regression analysis revealed an increasing trend in the diagnosis of enteritis over time.