A SURVEY OF DISEASES IN CAPTIVE RED WOLVES (*CANIS RUFUS*), 1997–2012

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Abstract: Conservation efforts to preserve the red wolf (*Canis rufus*) have been in progress since the 1970s through the U.S. Fish and Wildlife Service Red Wolf Recovery Program and the Association of Zoos and Aquarium's Red Wolf Species Survival Plan. An ongoing part of this project has been to monitor mortality trends, particularly to look for potential genetic conditions resulting from inbreeding given the small founding population of only 14 individuals. An initial survey was conducted in the 1990s but a comprehensive assessment of the population has not been done since then. This retrospective review evaluates mortality in the population from 1997 to 2012 through analysis of gross necropsy and histology records provided by cooperating institutions that housed red wolves during the time period of interest. Of the 378 red wolves that died during this 15-yr period, 259 animals had gross necropsy records, histology records, or both that were evaluated. The major causes of neonatal death were parental trauma, stillbirth, or pneumonia. Overall, juveniles had very low mortality rates with only 12 wolves aged 30 days to 6 mo dying during the study period. The most common cause of death within the adult populations was neoplasia, with epithelial neoplasms, carcinomas, and adenocarcinomas being the most common types reported. Gastrointestinal disease was the second most common cause of death, particularly gastric dilation and volvulus, inflammatory bowel disease, and gastrointestinal perforations. These findings are in stark contrast to causes of mortality in the wild population, which are primarily due to human-related activities such as vehicular trauma, gunshot, or poisoning. Overall, the captive population has few health problems, but an increase in inflammatory bowel disease in particular warrants further investigation.

Key words: *Canis rufus*, gastroenteritis, inflammatory bowel disease, neoplasia, pathology, red wolf.

INTRODUCTION

The distribution of red wolves (*Canis rufus*) once extended from the eastern to the south-central United States until populations were devastated by predator-control programs, habitat loss, and hybridization with coyotes (*Canis latrans*).[15,24] Following passage of the Endangered Species Act in 1973, the U.S. Fish and Wildlife Service established a recovery program to ensure the survival of the species. Over a 7-yr period more than 400 wild canids were captured in the red wolf's final range and evaluated through the recovery program for genetic purity, resulting in only 43 wild canids identified as probable red wolves.[7,28] From this group, 14 animals met the criteria developed to define the species and became the founding stock for the breeding program, with the goals of safeguarding the

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species through reproductive management and reestablishment of red wolf populations at select locations in the wild.[28] The reintroduction of red wolves born in the Red Wolf Species Survival Plan (RWSSP) began in 1987.[5]

Despite intensive conservation efforts, red wolves remain critically endangered. There are currently an estimated 100–120 red wolves inhabiting the red wolf recovery area, which spans five counties and encompasses 1.7 million acres in northeastern North Carolina.[5] Within the RWSSP population there are currently 207 red wolves, ranging from neonates to geriatric wolves, that are managed at 44 institutions.[29] These animals not only serve a role in the propagation of the species but also provide a means of monitoring disease prevalence and trends in a population based on limited founders.[23]

Wolves in general are affected by a variety of infectious and noninfectious diseases.[18] Published diseases in wild and captive red wolves include endometrial hyperplasia, pyometra, zinc-responsive dermatitis, patent ductus venosus, keratoconjunctivitis sicca, tick paralysis, and both ecto- and endoparasites.[1,3,4,6,8,9,11,17] Necropsy findings in the captive population was examined from 1992 to 1996.[2] This survey revealed that the most common causes of mortality in neonates were parental

Table 1. Summary of mortalities of captive red wolves (*Canis rufus*) over a 15-yr period, from 1997 to 2012, by age class and gender.

Age class	Male	Female	Unknown	Hermaphrodite	Total	Gross necropsy	Histology
Adults	135	133	0	0	268	174	143
Juveniles	10	2	0	0	12	4	3
Neonates	47	41	9	1	98	24	16
Total	192	176	9	1	378	202	162

trauma, parasitic pneumonia, and septicemia.[2] The two juvenile mortalities reported were both linked to congenital defects.[2] Cause of death in adult wolves was most often attributed to conspecific trauma, neoplasia, or gastrointestinal disease.[2] The survey indicated a healthy population with little evidence of deleterious effects as a result of a small founder population.[2]

Since the last publication there has not been a comprehensive mortality review of the captive red wolf population. The goal of this current retrospective study is to survey the population from 1997 to 2012 to determine if the primary causes of mortality have remained unchanged or if they have shifted over time, and if any new diseases have emerged.

MATERIALS AND METHODS

A review of the studbook revealed that 378 red wolves housed in captive institutions died between 1997 and 2012. Gross necropsy and histology reports were requested from all institutions that housed red wolves during this time. Gross necropsy reports were evaluated and classified by cause of death, if listed by the reporting clinician. Reports that did not contain an explicit cause of death were categorized as not stated. Cause of death was only classified as open if so designated by the clinician.

The histology findings were grouped based on the primary cause of death as assigned by the pathologist. Lesions were classified by body system; however, neoplasia, infectious disease, capture-related injury, and trauma were given their own classifications. Since causes of death were assigned by body system and etiology, some wolves were classified in more than one category. In cases where multiple causes of death were listed, the individual was counted in all of the appropriate categories.

Ancillary histologic lesions were also noted if available. Lesions were considered ancillary if they were benign processes, if they were not thought to be the primary contributor to cause of death even if they may have caused clinical disease, or if they were secondary to the processes leading to death. Gender and age at the time of death were recorded. Age was divided into groups corresponding with life stages: neonate (0–30 days), juvenile (30 days–6 mo), and adult (>6 mo) to allow for tabulation of common causes of mortality by age group.

RESULTS

From 1997 to 2012 a total of 378 red wolves died, ranging in age from 0 to 16 yr (Table 1). Records containing gross necropsy reports, histology reports, or both were obtained for 217 red wolves housed at 38 facilities. Two hundred and two animals had gross necropsy records, 162 had histology records, and 147 had both. An additional 42 cases had recorded case outcomes, although no gross necropsy or histology was performed. These red wolves were consumed by parents, stillborn, or missing (Table 2). There were 119 individuals that did not have records or for whom records were not provided. Of those wolves with no information provided, 80 were adults, 7 were juveniles, and 31 were neonates. Among the adult wolves, 96 cases had both a gross diagnosis and a histologic diagnosis. In 75% of these cases there was close agreement between the gross and histologic findings, while in 25% of the cases the two varied.

Gross necropsy findings

Neonates/juveniles: Gross necropsies were performed on 24 neonatal red wolves ranging in age

Table 2. Summary of 42 neonatal red wolves (*Canis rufus*) from 1997 to 2012 where no gross necropsy or histology was performed but case outcome was known. Summarized by cause of death and gender.

Cause of death	Male	Female	Gender unknown	Total
Consumed/assume consumed	10	17	2	29
Stillborn	7	2	1	10
Missing	2	1	0	3

Table 3. Primary cause of death of 24 neonatal red wolves (*Canis rufus*), from 1997 to 2012, based on gross necropsy. Characterized by cause of death and gender.

Primary cause of death	Male	Female	Hermaphrodite	Total
Trauma	9	4	0	13
Open	1	5	0	6
Atresia ani	0	1	1	2
Rectovaginal fistula	0	1	0	1
Stillborn	1	0	0	1
Not stated	1	0	0	1

Table 4. Primary cause of death of 175 adult red wolves (*Canis rufus*) from 1997 to 2012 based on gross necropsy and categorized by gender and cause of death.

Cause of death	Males	Females	Total
Neoplasia	17	25	42
Gastrointestinal disease	16	22	38
Not stated	13	11	24
Open	13	8	21
Renal disease	6	5	11
Trauma	3	7	10
Cardiac disease	5	4	9
Reproductive disease	0	8	8
Capture-related	6	1	7
Respiratory disease	2	2	4
Musculoskeletal disease	4	0	4
Hematopoeitic disease	2	1	3
Ophthalmic disease	2	0	2
Sepsis	1	1	2
Anesthesia-related	2	0	2
Neurologic disease	0	1	1
Autoimmune disease	1	0	1

from 0 to 13 days old (Table 3). Thirteen of the 24 neonates died due to trauma. Six of the 13 were traumatized by the dam or sire, five drowned when their den flooded after a rainstorm, and two died from trauma of unknown cause. Four juvenile red wolves, ranging in age from 38 to 117 days, had gross necropsies performed. One died as a result of conspecific trauma; the other three wolves had no cause of death listed on their gross necropsy report.

Adults: One hundred and seventy-five adult red wolves, 85 males and 90 females, had gross necropsies performed (Table 4). The two most common causes of death were neoplasia ($n = 42$) and gastrointestinal disease ($n = 38$). When gastrointestinal disease was examined further, the most common lesion was gastric dilation and volvulus (GDV), which resulted in the death of 16 wolves. Nine wolves had gastric or intestinal ulcerations and/or perforations. A history of long-term steroid use was reported in one of the nine. Other gastrointestinal conditions included gastroenteritis ($n = 7$), hepatic disease ($n = 4$), intussusception ($n = 2$), protein-losing enteropathy ($n = 2$), and gastrointestinal parasitism ($n = 2$).

Other leading causes of mortality in adult red wolves included renal disease ($n = 11$), trauma ($n = 10$), cardiovascular disease ($n = 9$), capture-related mortality ($n = 7$), and reproductive disease ($n = 8$). Nine of the 11 wolves with renal disease had chronic renal disease, but in two instances renal failure was associated with pyometra. Trauma resulting in death was primarily due to conspecific aggression ($n = 7$). However, one wolf was crushed when a den collapsed and one wolf died following dart-induced laceration of the femoral artery. Cardiovascular abnormalities included cardiomyopathy ($n = 3$), cardiomegaly ($n = 2$), and congestive heart failure ($n = 2$), one of the latter due to heartworm infection. In two instances, the type of cardiac abnormality was not listed.

Capture-related deaths resulted from myopathy ($n = 4$) or hyperthermia ($n = 3$). Death attributed to reproductive disease was limited to females with pyometra ($n = 8$). Three males were cryptorchid, but these lesions did not contribute to mortality.

Other less frequent causes of death included respiratory disease, musculoskeletal disease, ophthalmic disease, neurologic disease, autoimmune disease, age-related disease, or sepsis (Table 4). In 45 cases the cause of death was not defined; it was either listed as "open" or no cause was listed in the reports.

Histologic findings

Neonates/juveniles: Histology was performed on 16 neonatal red wolves, 4 stillborn and 12 viable. Of the four stillbirths, two had evidence of trauma and two had interstitial pneumonia. The most frequent cause of death of the viable neonates was respiratory disease, specifically pneumonia, which was a primary contributor to mortality in five wolves (31%). Two had interstitial pneumonia, one had bronchopneumonia, one had bronchointerstitial pneumonia, and in one case the type of pneumonia was not stated. In two of the cases sepsis was also present. Pneumonia was attributed to staphylococcal infection in one red wolf, while the underlying etiology was undetermined in the other four instances of pneumonia. Lesions consistent with trauma were the primary histologic finding in two cases (12.5%). The remaining deaths were attributed to congenital atresia ani ($n = 2$), ulcerative

Table 5. Primary cause of death of 143 adult red wolves (*Canis rufus*) from 1997 to 2012 based on histology and categorized by cause of death and gender.

Cause of death	Males	Females	Total
Neoplasia	20	23	43
Gastrointestinal disease	8	16	24
Renal disease	11	8	19
Infectious disease (bacterial, viral, fungal)	3	10	13
Shock	5	7	12
Reproductive disease	0	9	9
Cardiac disease	5	3	8
Open/undetermined	6	2	8
Age-related changes	5	2	7
Trauma	2	5	7
Parasitic disease	4	2	6
Respiratory disease	3	3	6
Capture-related	4	0	4
Neurologic disease	1	2	3
Sepsis	0	2	2
Hematopoeitic disease	2	0	2
Musculoskeletal disease	2	0	2
Autoimmune disease	1	0	1

pododermatitis ($n = 1$), hepatic disease ($n = 1$), or unknown causes ($n = 1$).

Of the three juvenile red wolves with histology reports, one had an apparent heart defect based on passive congestion in the liver and lungs. This wolf also had abnormal histologic architecture of the myocardial fibers which was presumed to be congenital based on signalment and history. Shock was the cause of death in one juvenile and the cause of death was not determined in the third wolf in this age category.

Adults: Among the 143 adult red wolves that had histology performed there was a wide variety of lesions in multiple body systems (Table 5). The primary cause of adult mortality was neoplasia (30%). There were multiple types of neoplasms, the most common being carcinoma or adenocarcinoma ($n = 26$), and lymphoma ($n = 5$). There were also reports of pheochromocytomas, osteosarcomas, fibrosarcomas, granulosa cell tumors, sarcomas, lymphosarcomas, nerve sheath tumors, sertoli cell tumors, histiocytomas, and adenomas. Neoplastic processes involved a wide array of organ systems but were most commonly noted in the gastrointestinal system, genitourinary system, and respiratory tract. There was a higher prevalence of neoplastic lesions with age, with 80% of animals being older than 10 yr of age, 14% being between 5 and 10 yr, and 6% being between 1 and 5 yr of age.

Gastrointestinal lesions directly caused or contributed to mortality in 16.8% of adults that had histology results. The most common lesions were chronic gastritis and/or chronic enteritis ($n = 8$). Four wolves had lymphoplasmacytic and eosinophilic enteritis, two had lymphoplasmacytic enteritis, and two had necrotizing enteritis. Gastrointestinal perforations were present in six wolves; four perforations were caused by full-thickness ulcerations, one was due to a penetrating foreign body, and one was due to neoplasia. Lesions consistent with GDV or mesenteric torsion were found in five wolves. Additional lesions included intussusception ($n = 2$) and included hepatic microvascular dysplasia ($n = 1$). Another 37 wolves (25.9%) had gastrointestinal lesions, which may not have contributed to death, 22 of which were gastritis and/or enteritis (59%).

Renal disease was the primary cause of death in 13.3% of adult wolves. Renal lesions included chronic interstitial nephritis ($n = 11$), glomerulonephritis or glomerulosclerosis ($n = 7$), chronic tubulointerstitial nephritis ($n = 1$), nephrosclerosis ($n = 1$), glomerulonephritis or glomerulosclerosis ($n = 7$), amyloidosis ($n = 1$), and acute renal tubular necrosis ($n = 1$). An additional 51 wolves had renal lesions that likely did not contribute to death, most commonly nephritis/nephrosis or glomerulopathy.

Shock was responsible for the death of 8% of adult wolves. In the majority of cases the cause of shock was unknown, but, when determined, included GDV, trauma, and potential drug reactions. Lesions attributed to trauma were the cause of death in seven wolves.

Reproductive disease was the primary cause of death in 6.3% of the wolves, all of which were females. Suppurative endometritis was the primary lesion in eight of these wolves. Nonfatal reproductive lesions were present in 14 males and 12 females. In males, the most common findings were testicular or prostatic atrophy ($n = 7$) and in females, cystic endometrial hyperplasia was the most common finding ($n = 7$).

Death was attributed to cardiac disease in 5.6% of the wolves. Specifically, epicarditis/endocarditis ($n = 2$), heart failure secondary to myocardial and valvular fibrosis ($n = 2$), acute myodegeneration/myonecrosis ($n = 3$), and dirafilariosis ($n = 1$). Nonfatal cardiac lesions were noted in 29 wolves, with age-related endocardiosis being the most common.

Seven wolves (4.9%) had only incidental age-related changes noted at time of death. Six wolves had respiratory lesions as the primary cause of

death. In these six wolves, the lesions were attributed to *Dirofilaria* in four cases, ascarid migration in one case, and *Paragonimus kellicotti* in one case.

In seven wolves the cause of death could not be determined histologically. There were also instances of neurologic lesions, hematopoietic lesions, musculoskeletal lesions, autoimmune disease, and sepsis, but these lesions only affected a small number of the population (2% or less).

Parasitic disease contributed to mortality in six wolves. Infectious disease was implicated in 13 red wolves (9%), of which bacteria were the primary pathogens in 11 of these cases and resulted in either bacterial pneumonia or pyometra, with a single case resulting from an infected surgical incision. Suspected viral disease and fungal disease were each considered the causative agent in the death of a single wolf, but the agents were not further characterized.

DISCUSSION

In comparison to previous findings within the captive red wolf population, there were some notable changes in mortality trends. Earlier reports showed a high prevalence of parasitism among neonates, with 86% having evidence of internal parasitism, particularly ascarids.[2] No neonates within this study showed evidence of internal parasitism. The dramatic decrease in prevalence of parasitism is likely due to increased diligence in prophylactic deworming at facilities housing red wolves based on previous recommendations.[2]

Another major finding within neonates in the previous review was footpad ulcerations that were suspected to have led to sepsis.[2] Only a single neonate in this current review was noted to have footpad ulcerations, indicating that appropriate substrate changes have been made to reduce the incidence. However, it is unknown whether or not neonates that were consumed by the parents had footpad lesions, thus the prevalence may be understated in this report.

The major causes of death within the neonatal population were stillbirths or trauma, the latter resulting from factors either related to the enclosure or from the parents. Many of the stillbirths did not have tissue samples collected for histologic analysis, and the cause of death was undetermined. A total of 29 pups were consumed by either the sire or the dam. Fourteen of these pups (48%) had the same sire and dam and represented that pair's first and second litters. This same pair subsequently produced a third litter of two pups that were not consumed. It is possible that the larger litter sizes or parental inexperience may have contributed to the initial high mortality. The female was never paired with another wolf, but the male successfully sired pups with three other females with no incidences of parental trauma.

In the previous evaluation of mortality within the red wolf population, there was a very low mortality rate among juveniles.[2] This trend persisted within the time span of this current retrospective study. Of the 378 deaths, only 12 (3%) were between 30 days and 6 mo of age, supporting the previous finding that there is substantially increased survivability after the first month of life.[2]

Consistent with previous evaluations of the captive red wolf population, the most common cause of death in adult red wolves was neoplasia. Epithelial malignancies were the most commonly diagnosed types of cancer. This diverges from the prior retrospective assessment, which identified lymphosarcoma, carcinoma, and osteosarcoma as the most prevalent types of neoplasm in red wolves.[2] Lymphosarcoma and osteosarcoma were less commonly diagnosed in the current study. Multiple types of neoplasms have been described in other wolf species including fibrosarcoma, extraosseous osteosarcoma, osteogenic sarcoma, and ovarian tumors in maned wolves (*Chrysocyon brachyurus*); tonsillar squamous cell carcinoma in a polar wolf (*Canis lupus arctos*); and mammary neoplasia and nasal carcinomas in Mexican gray wolves (*Canis lupus baileyi*).[12,19,20,23,25–27] In domestic dogs, lymphoma is the second most common neoplasm, whereas it ranked third in prevalence in this red wolf population.[14] Other types of neoplasia that are frequently diagnosed and treated in domestic dogs, including mast cell tumors and hemangiosarcomas, were completely absent or not identified in this group. This may indicate that these types of tumors do not affect red wolves with the same frequency as they do domestic dogs.

The second most common cause of mortality in adult red wolves was gastrointestinal disease, found in 21.7% of wolves at gross necropsy and 16.8% of wolves on histologic examination. The two most common gross gastrointestinal lesions were GDV and gastrointestinal perforations. The underlying etiology of GDV in domestic dogs is not fully known but risk factors include deep-chested body conformation, exercise with a distended stomach, and ingestion of large amounts of food or water at a single time.[10] Given these risk factors, it is possible that the body conformation

of the red wolf contributes to the incidence of GDV in this species. Furthermore, in some cases, GDV occurred after the animals were captured, and was likely preceded by periods of intense evasive exercise.

Histologically the most common clinically important gastrointestinal lesions in adult wolves were idiopathic lymphoplasmacytic and/or eosinophilic gastritis and/or enteritis resembling the inflammatory bowel disease (IBD) seen in dogs and cats. Additionally, 37 adult red wolves had inflammatory gastrointestinal changes noted as nonfatal lesions. This is a shift from the previous retrospective evaluation in this species, which found lesions consistent with parvovirus infection to be the most frequent enteric histologic finding.[2] The lack of parvovirus-like lesions may indicate that facilities are maintaining better vaccination and quarantine protocols or that there is decreased exposure to the disease.

IBD is a broad term that encompasses a group of disorders that are characterized by chronic inflammation of the intestinal tract.[13] There are a variety of disease processes that may be associated with IBD, including parasitism and food allergies, but the etiology is unknown.[13] The increased prevalence of inflammatory gastrointestinal lesions warrants further research to determine the underlying cause and contributing factors.

Red wolves have one of the highest rates of uterine disease among wild canid species, second only to the African painted dog (*Lycaon pictus*).[4,16] This retrospective study found that 8.8% of necropsied female wolves had pyometra listed as the primary cause of death. Among females that had histology performed, 11.8% had either suppurative endometritis or severe cystic endometrial hyperplasia (CEH). An attempt to treat pyometra in red wolves in an effort to preserve reproductive capacity has been documented.[3] The risk of developing CEH increases in both incidence and severity with the use of synthetic progestin contraceptives such as melengestrol acetate.[22] Although these birth control measures are highly effective in preventing pregnancy, the risk of uterine disease likely outweighs the benefit. Other methods of limiting unwanted breeding should be considered, such as ovariohysterectomizing females who are not genetically valuable to the population as a whole. While physical separation of males and females is another option, repeated barren cycles may predispose female red wolves to uterine disease.[4]

The prevalence of infectious diseases in the study population was low. There were no cases of parvovirus or canine distemper virus infection, indicating that vaccination protocols and other control measures have been effective in preventing these particular diseases within the population.

Given the small founder population, genetic diseases are of particular concern. Three adult males (3.3%) were noted to be cryptorchid on gross examination. Since cryptorchidism is considered hereditary in domestic canids, it is recommended that affected individuals not be used for breeding purposes.[21] The previous retrospective study showed evidence of progressive retinal atrophy (PRA) in three wolves. Since PRA is genetically linked in domestic dogs there was concern that this may also be the case in red wolves.[2,30] In the current study, only one individual was identified as having PRA, suggesting that potentially selective breeding has prevented further prevalence of this condition, or that it may not have the same genetic link as is noted in domestic species.

There was a high level of agreement between the gross diagnosis and the primary histologic findings. Based on the increasing incidence of gastrointestinal disease it is particularly important to focus on the gastrointestinal system when conducting a gross necropsy and collect multiple samples from the entire length of the gastrointestinal tract in addition to collecting a complete tissue set for histologic evaluation.

Despite the broad scope of this retrospective review, there are some inherent limitations. Histologic examination was performed by numerous pathologists, increasing interuser variability in the histologic interpretation. Furthermore, in an effort to include the most data, all individuals that had either a gross necropsy report or histology report were included, even if tissues were severely autolyzed. Records were requested from all facilities that housed red wolves during the time period of interest; however, not all facilities responded, and several animals did not have records, so the prevalence of disease in the population may be underestimated.

The findings of this study are at times in contrast to the common causes of mortality in the wild red wolf population. In the previous 13 yrs 248 wild red wolf mortalities have been documented (Bartel, pers. comm.). Of these individuals, only 11.7% were attributed to health-related issues (Bartel, pers. comm.). In comparison, 63.1% of wild red wolf mortalities

were attributed to human-related activity, including vehicular trauma, poisoning, gunshot, illegal take, or private trapping (Bartel, pers. comm.). This underscores the very different challenges in managing in-situ and ex-situ populations of the same species.

CONCLUSIONS

The findings of this retrospective review corroborated the previous examination of this population in that neoplasia and gastrointestinal disease were considered to be the most common causes of mortality in adult red wolves, and that wolves are less likely to die during the juvenile stage. In contrast, there were some shifts including reduced prevalence of parasitism and ulcerative pododermatitis in neonates. Overall the prevalence of infectious disease was low and there were no findings consistent with parvovirus or canine distemper virus within the study population.

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