

Significant Diseases of Two Very Aged Red Foxes (*Vulpes vulpes*, Demarest 1820)

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Abstract

Wild mammal survival rarely approaches ages that are possible in genomic terms. Thus, sheltered environments present unique opportunities to study wild mammal aging and compare aging-related disorders to those of similar domestic animals. We evaluated two aged red fox vixens that had lived in a park setting where they were not caged, and were provided an environment that reflected a natural setting as closely as possible, while providing for proper nutrition and health care. During their 14th year, both foxes developed symptoms suggesting orthopedic and renal deterioration. After diagnosis, monitoring, and humane euthanasia,

postmortem evaluation revealed degenerative joint and renal disease, each closely resembling organ-specific pathology of aged domestic dogs.

Based on evaluation of these foxes and other recent reports, we hypothesize that underlying mechanisms for late life pathological changes reflect broadly and deeply conserved response capacities and not artificial environments, provided that shelter, nutrition, and health care are adequate to minimize stochastic mortality that prevails in the wild.

Keywords: Aging, hip, kidney, osteoarthritis, red fox, shoulder, *Vulpes vulpes*

Introduction

Background

The red fox (*Vulpes vulpes [fulva]*, Demarest 1820) is highly successful, having the largest global distribution of any extant terrestrial carnivore (Henry 1996; Cypher 2003). Its dispersal patterns appear related to population density, interspecies competition, and habitat change (Allen and Seargeant 1999). Adult size is variable with mature body weight 3.0-8.0 kilograms, but on average it is the largest among North American foxes (Cypher 2003). Longevity of 8.6 years in the wild has been reported for a tagged red fox, but in free-living populations, survival as long as 6 years is uncommon (Allen and Seargeant 1999; Storm et al. 1976; Tuller 1983). Since survival in the wild rarely approaches true species limits of longevity, elucidating the aging process in wild species remains a very difficult task. Considering available observational methods, establishing control populations of "normal" free-roaming subjects for comparative studies is nearly impossible because of the natural delay between biological onset of many late life diseases and onset of overt symptoms, and because of the ability of wild mammals to disguise pain and symptoms of organ disease. Thus, the most pressing need is to report all species-related

aging observations to build a database from which to clarify late life diseases in wild mammals. In this regard, we report examination of shoulder and hip joints, and kidneys, of two red fox vixens that were euthanized during their 14th year because of orthopedic and renal disease. The observations, along with other recent studies, contribute to expanding our understanding of the mammalian aging process.

Life histories

The two females (vixens), named Devon and Ember (not siblings), were born in the spring of 2000. They were habitat-reared and maintained together. They were fed a natural diet consisting of deer meat and small rodents, occasional rabbits, fruits and vegetables, and they regularly caught small rodents and insects such as cicadas. They resided at Wolf Park, Battle Ground IN, in an enclosure measuring 60 x 40 feet, with ladders and decks for climbing and jumping, thus allowing natural fox behavior. They were vaccinated for rabies, distemper, leptospirosis, parvovirus, coronavirus, adenovirus, parainfluenza virus, and lyme disease, and were monitored daily. Health history of each fox was unremarkable until late life.

A few weeks prior to humane euthanasia in their 14th year, both foxes exhibited signs of reduced activity and declining range of joint motion, resulting in end-of-life maintenance indoors. Biochemical evaluation indicated progressive renal failure in both foxes (Table 1). Following humane euthanasia, postmortem examinations were done by the attending veterinarian as a part of normal health care practices. The foxes were not part of a research study, and Institutional Animal Care and Use Committee clearance was not required.

Materials and Methods

Tissue harvest and processing

Gross postmortem examination of both foxes revealed pale yellow, rough-surfaced kidneys that were not contracted or nodular. The left kidney from Ember was preserved in formalin and sent to the consulting pathologist for routine histological evaluation, to parallel routine veterinary clinical practices.

Harvest of shoulder and hip joints was done *en bloc*, followed by freezing at -4°C. Other remains were buried with ceremony, according to Park practices. The frozen bone specimens were transferred to the Illinois State Museum Research and Collections Center, Springfield IL. Bone processing was continued by trimming soft tissue and incubating in hot

water (40-45°C) to macerate remaining non-ossified tissue by bacterial action, with filtering and water replacement as needed. Following several weeks' incubation, soaking in 15-20% ammonia solution promoted further removal of fat from bone. A final brushing step in detergent was done to remove tightly adhered exuded fat. Following clearing, bones were rinsed in tap water and dried. Evaluation of joint components included scapular, humeral, acetabular, and femoral articular surfaces and margins, and periarticular structure. Lumbar vertebrae 5-7 and the sacrum were available from Ember. All harvested bone components were photographed and descriptions recorded.

Results

Renal disease

Renal histopathology (Ember) yielded diffuse, moderate-severe, chronic, glomerular and tubular, calcinosis and interstitial fibrosis (Table 1, Fig 1).

Scapular glenoid

Glenoid fossae were bordered or impinged by encircling articular margin / periarticular prominence, rimming, or osteophytes that were severe in Devon and mild in Ember. Glenoid articular surfaces (Devon) revealed circular caudolateral features (3mm, left side affected more severely) characterized by loss of articular

bone and exposure of thickened underlying trabeculae (Fig 2). Ember was affected similarly but less severely. Both foxes also revealed right glenoid pinpoint depression caudolateral (Devon) or craniocentral (Ember), consistent with focal ossification failure.

Proximal humerus

Proximal humeral articulations were bordered or impinged by encircling articular margin/periarticular prominence, rimming, or osteophytes. The features were severe in Devon (Fig 3) and milder in Ember. Both intertubercular (bicipital) grooves revealed severe osteophytes in Devon (Fig 4), while similar lesions in Ember were less severe. The latter features are compatible with degrees of co-morbid biceps tendonitis. Ember also revealed a 1x3 mm mid-lateral articular surface defect on the left humerus.

Acetabulum

Acetabular fossae of both foxes revealed bone formation suggesting enthesiophytes. The new bone formation was more severe in Devon, impinging the dorsomedial articular surface bilaterally (Fig 5). The medial acetabular articular margins were prominent in both foxes, while Devon had small osteophytes at each caudal acetabular margin.

Proximal femur and spine

The dorsal femoral necks revealed very mild filling with new bone (Devon), and prominent femoral head articular margins (Devon and Ember, with greater severity in Devon). Ember had severe spondylosis deformans of cranial and caudal aspects of lumbar vertebrae 6 and 7, and cranial sacrum.

Discussion

Behaviors and osteoarthritis

The red fox hunts small prey by pouncing and pinning the prey with its forefeet, killing it with a bite. Suddenly attacking small prey from brush cover also is a common behavior. Somewhat larger prey (rabbits) are stalked and attacked with jumping or sprinting behaviors (Scott 1943). Other normal fox behaviors include brisk walk as the normal gait; trotting up to several kilometers and jumping to about 4 meters; occasional swimming or climbing; and active defense of a home range (Pasitschniak-Arts and Pasitschniak-Arts 1996). At Wolf Park, very similar behaviors (especially in young foxes) include jumping from heights of 1.3-2.0 m; climbing fences, play-ladders, or trees; pouncing on insects such as cicadas; and playing on a trampoline.

Given an active lifestyle and carnivorous behaviors, significant prevalence of arthropathy might be expected in the uncommon event that a free-living fox survives to advanced age. Obesity is a well-known risk for osteoarthritis in several mammalian species (Anderson and Felson 1988; Davis *et al*, 1988; van Saase *et al*, 1988; Bendele and Hulman 1991; Popovitch *et al*, 1995; Lawler *et al*, 2008), but obesity is unusual in wild canids. In the foxes we evaluated, only seasonally-expected fluctuations in body condition were observed, without obesity at any time during life. Thus, environmental and nutritional influences seem insufficient to explain the disease observations. The latter is interesting, considering the similarities to the parallel conditions among elderly domestic dogs.

Renal disease

The similarity of the fox renal morphology to late-life renal disease in domestic dogs prompts hypotheses that (a) soft organ diseases of aged Canidae may be more universal consequences of simple chronology than has been supposed; and (b) immediate environments may be correspondingly less influential provided that nutrition, shelter, and health care are adequate. Additional late life biochemical studies of wild canids that live to species-possible ages should

be conducted and published, to explore more completely the chronological prevalence of diseases across related individuals in non-domestic populations.

Joint disease – shoulder

In a cross-sectional study of 88 randomly-selected domestic dogs (mean age 8.2 years, range 1-12), shoulder radiography revealed primary age-related osteoarthritis in 31% of the group (mean age 10.2 years)(Ljunggren and Olsson 1975). A cross-sectional study of skeletal remains of 16 adult raccoon dogs (*Nyctereutes procyonoides*) revealed 100% prevalence of the same glenoid features that were observed in the two vixens, with severity ranging from near-normal to mild osteoarthritis, while 81% of proximal humeral articulations also reflected the fox observations (Lawler *et al*, 2012). Recently, lesions of scapular glenoid articular cartilage were related spatially to underlying pathology of articular bone in 7 gray wolves (ages 5-19 years), and the nature and spatial distribution of the osteoarthritis changes were again the same as the foxes and raccoon dogs (Lawler *et al*, 2016).

A longitudinal study of 149 domestic dogs revealed 53% radiographic prevalence of age-related shoulder osteoarthritis at mean age 13.8 years

(Morgan *et al*, 1987). In another longitudinal study, 43 Labrador retrievers had more radiographic shoulder osteoarthritis at age 8 years than elbows, hips, and stifles of the same dogs (Kealy *et al*, 2000).

Joint disease – hip & spine

One left femoral head lesion (Devon) is compatible with an early circumferential femoral head osteophyte (Szabo *et al*, 2007). The red fox osteoarthritis-related pathology of acetabula and proximal femoral articulations mimic observations of domestic dogs (Lawler *et al*, 2008) and raccoon dogs (Lawler *et al*, 2012). A report of femoral head radiographic pathology in a gray wolf (*Canis lupus lupus*) described very similar features (Lawler and Evans 2016). The intra- and extra-articular spatial locations of these features are consistent across canids examined to date, with the expected age-related variation in severity.

Spondylosis refers to osteophyte formation around cranial and/or caudal vertebral end plates, secondary to degenerative disease of the adjacent intervertebral disk (Morgan and Biery 1985). The intervertebral disk space may be narrowed and adjacent vertebrae become unstable, prompting osteophyte reaction. Older large domestic dogs develop spondylosis more frequently, but smaller dogs

also are affected. Some affected dogs experience pain and difficult movement, while others remain relatively asymptomatic (Prada 1993). We are not aware that this pathology has been investigated in red foxes, but the strong similarity between fox and other canid features again is striking.

Studies of domestic dogs demonstrate that radiography significantly underestimates diarthrodial joint pathology (Morgan *et al*, 1987; Runge *et al*, 2008; Lawler and Evans 2016), and radiographic diagnosis of joint pathology in other canids introduces these same limitations (Lawler *et al*, 2016). We suggest that comparative joint biology studies of Canidae have been impeded by (a) limitations inherent in most imaging modalities; (b) hazards of anesthetizing wild animals for examination; (c) relative infrequency of highly detailed postmortem study of wild mammal diarthrodial joints.

Integrative joint development and function

Studies of morphological integration examine relationships among anatomical structures (Cheverud 1982, 1995, 1996). Common functional attributes, such as the shape of the proximal humerus and scapular glenoid fossa, lead to functional integra-

tion with muscle actions that are necessary for proper function, implying common developmental influences during formation and growth (integration). These influences may be hormonal, or they may relate to interaction of developing tissues.

Integrated structures evolve together because of correlated inheritance and shared influence from natural selection. Functional and developmental morphological integration occur at the individual level, and evolutionary integration occurs at the population level (Cheverud 1982, 1995, 1996).

Integrated traits are expected to exhibit a significant correlation among traits within the anatomical module, based on their shared influences (Cheverud 1982, 1995, 1996; Hallgrímsson *et al*, 2002). Morphological integration studies of mammalian scapulae have examined the developmental, functional, and evolutionary structure of the scapular glenoid and blade (Young 2004, 2006, 2008). Scapula studies of seven primate species (Young 2004) and six mustelid species (Norman 2016) identified significant integration within the glenoid, as well as within the blade and acromion. Locomotion influences on integration were observed in 17 primate species (Young 2008). Developmental influences of the *Hoxc6* gene on glenoid

(Olivier *et al*, 1990); *Emx2* gene influencing the scapula blade (Pellegrini *et al*, 2001); and *Pax1* gene influencing the scapular blade and acromion (Timmons *et al*, 1994; Wilm *et al*, 1994), help to elucidate the genomic integration of these developmental and functional regions. It follows that associated soft tissue structures should be governed under common developmental and functional influences.

Further evaluations of integrated diarthrodial joint components, based on phylogeny and functional morphology, should further clarify why degenerative joint pathology tends to occur at nearly identical foci within the same joint, across species.

Conclusions

It is clear that aging-related osteoarthritis is not unique to the domestic dog and occurs among other Canidae, in similar joint-specific spatial patterns. Primary influences on expression of late life diseases among canids appear to reflect (a) species-related achievable longevity; (b) the balance among disease onset and the subsequent time that symptoms develop to be observed; and (c) genome-mediated responses to insults that appear to be conserved phylogenetically.

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Table (1): Biochemical documentation of progressive renal disease in two elderly female *Vulpes vulpes*

Ember

	<u>Urea nitrogen (mg/dl)</u>	<u>Creatinine (mg/dl)</u>	<u>Phosphorus (mg/dl)</u>	<u>Hematocrit (%)</u>
8/29/2014	222	4.7	16.5	18
7/23/2014	201	5.3	13.4	30
6/14/2014	154	5.0		35
5/28/2014	136	5.0	11.7	21
5/15/2014	149	4.6		34
5/01/2013	68	1.7		48
7/11/2012	64	1.1	3.9	48
2/27/2011	59	1.0		46
1/30/2010	26	0.6	2.6	47

Devon

	<u>Urea nitrogen (mg/dl)</u>	<u>Creatinine (mg/dl)</u>	<u>Phosphorus (mg/dl)</u>	<u>Hematocrit (%)</u>
7/22/14	195	7.5	13.9	28
6/14/14	162	5.3		36
5/15/14	182	4.7		39
9/11/13	83	1.3	4.4	39
5/01/13	65	0.8		49
7/11/12	48	0.9	3.9	43
7/27/11	46	0.7		46
3/05/11	32	0.7	3.6	57
8/24/10	48	0.7		37

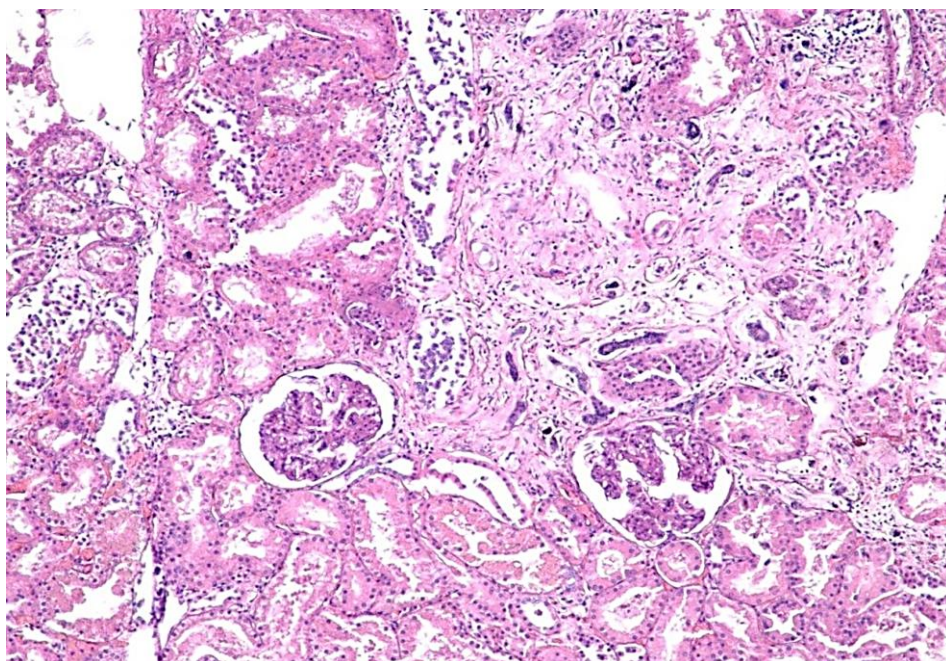


Fig (1): Ember, left kidney. Nephropathy, glomerular and tubular, dystrophic with calcinosis, chronic, moderate-severe, diffuse (H&E).

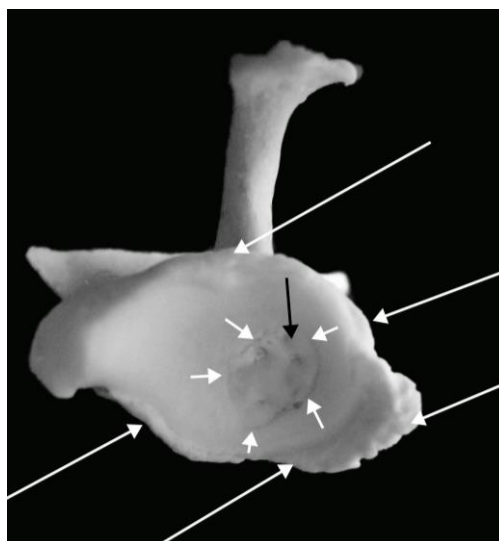


Fig (2): Devon, left scapula glenoid cavity. Severe encircling osteophytes (long white arrows); Bone loss mid-caudal fossa (short white arrows); Exposed thickened subarticular trabeculae (black arrow)

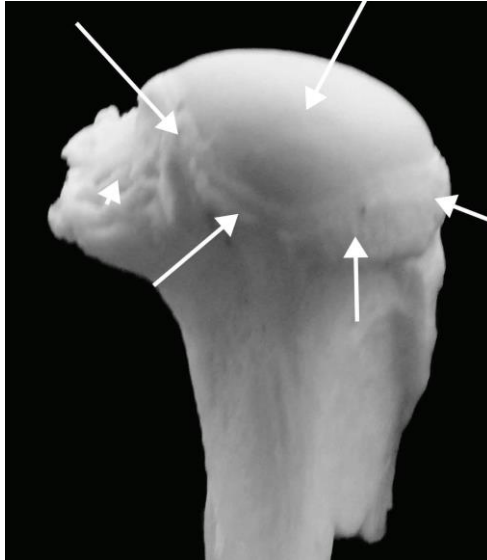


Fig (3): Devon, right proximal humerus, caudolateral view. Normal caudolateral articular surface (long white arrow); Severe encircling articular margin & periarticular osteophytes impinging margin of articular surface (short white arrows); osteophytes on lesser tubercle (white arrowhead).

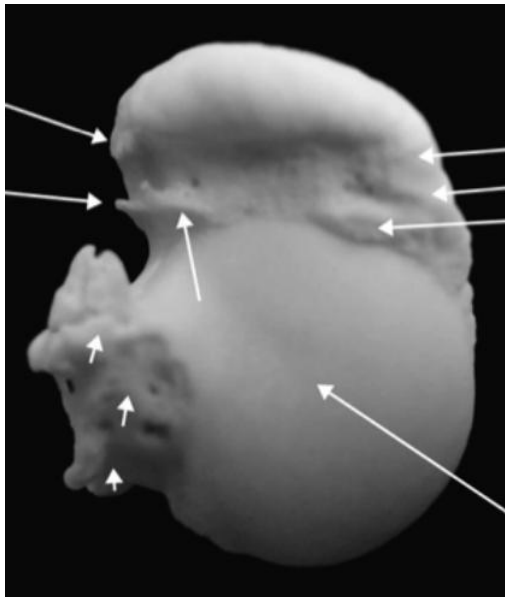


Fig (4): Devon, right proximal humerus, articular surface, dorsal view. Normal articular surface (long white arrow); Osteophytes on lesser tubercle (white arrowheads); Severe osteophytes medial & lateral margin & floor of intertubercular (bicipital) groove (short white arrows).

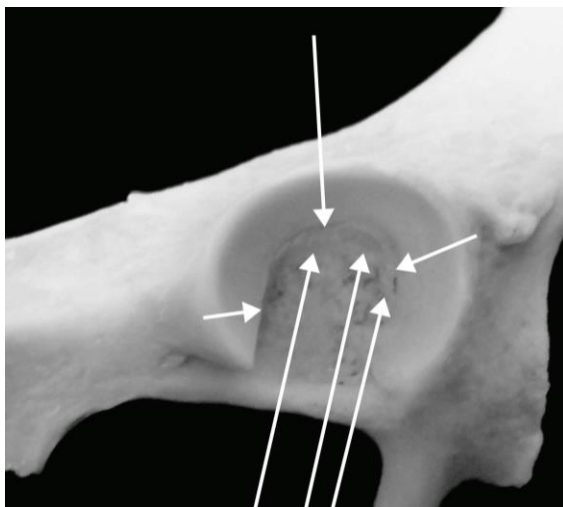


Fig (5): Devon, right acetabulum, lateral view. Severe new bone craniodorsal fossa, probable enthesiophytes (long white arrows); Impinging of medial articular surface (medium arrow); Prominent medial articular margin (short white arrows).