PACHYDERMOPERIOSTOSIS-LIKE DISEASE IN CAPTIVE RED RUFFED LEMURS (VARECIA VARIEGATUS RUBRA)

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ABSTRACT

Pachydermatoperiostosis, a rare form of hypertrophic osteoarthropathy, is of unknown etiology and previously thought limited to humans. The only periosteal reaction previously reported in prosimians is related to renal disease. Notation of hypertrophic osteoarthritis in three prosimians led to recognition that this was the first non-human documentation of the disease. Three related red ruffed lemurs (*Varecia variegatus rubra*) had diaphyseal periosteal reaction classic for hypertrophic osteoarthropathy. Workup was negative for known underlying causes and for the secondary hyperparathyroidism which produces bone alterations in black *Eulemur macao,* black and white *Varecia variegatatus varigatus* and ringtail *Lemur catta* lemurs. Recognition of facial coarsening allows identification of the primary form of hypertrophic osteoarthropathy, categorized in humans as primary hypertrophic osteoarthropathy. This is the first recognition of the phenomenon in the order primates, exclusive of humans, and represents a new model for this rare disease.

Key words: *Varecia variegatus rubra*, hypertrophic osteoarthropathy, pachydermatoarthritis, periosteal reaction, lemur, bone pathology

Primary hypertrophic osteoarthropathy (PHOA), also referred to as pachydermoperiostitis and pachydermoperiostosis, is a rare disease, previously reported only in humans.[1-5] While elimination of secondary causes for hypertrophic osteoarthropathy is part of the diagnostic approach, it is often the coarsening of facial features that suggests the diagnosis of the primary form.[6] It presents in humans as

autosomal dominant with symptoms usually appearing around puberty.[1,4,7] Recognition of the phenomenon in this group of related lemurs suggests the availability of this species as a new model for this baffling disease.

Prosimians, order primates, rarely develop bone pathology[8,9] which, when noted, presents as irregular overgrowth of the outer layer of bone cortex, referred to as periosteal reaction. Previously reported cases in black (*Eulemur macao*) and black and white (*Varecia variegatus variegatus*) lemurs[10,11] have been related to the secondary hyperparathyroidism of renal disease and, in ringtail lemurs (*Lemur catta*), to nutritional secondary hyperparathyroidism.[11]

The findings in red ruffed lemurs (*Varecia variegatus rubra*) support a different diagnosis. Examination of the character and distribution pattern of periosteal reaction in an adult female identified a phenomena well recognized in mammals, hypertrophic osteoarthropathy (HOA).[12-14] Most HOA is secondary, a complication of intrathoracic pathology and liver disease.[15-19] However, after an exhaustive workup identified none of the known causes in the propositus, one of us (DN) commented that her subsequently-affected daughters had the same facial coarsening. This led to the recognition that these lemurs actually represented the first animal model of the primary form of HOA.[1-3,20]

MATERIALS AND METHODS

Affected lemurs underwent full clinical, laboratory and radiologic evaluation to characterize the underlying disease process and to seek an effective approach to its treatment. Autopsy was performed on the propositus.

RESULTS

Case 1

An 8 year old, 3.5 kg, female red ruffed lemur (Varecia variegatus rubra, lemur 1, propositus) was hand-restrained for examination to evaluate reported lethargy and hind leg stiffness. Physical examination was unremarkable, with exception of decreased muscle mass. Complete blood count (CBC) and serum chemical assays were within normal species range,[21] with exception of globulin [3.6 gm/dl, ISIS (International Species Inventory System) range = 1.9 ± 0.7 gm/dl] and albumin (3.2 gm/dl, ISIS range = 5.7 ± 0.8 gm/dl). Evaluation 6 weeks later revealed decreased total red blood cell count (RBC) (7.8 x 10^6/UL, ISIS range 9.31 ± 1.01 x10^6/UL), and phosphorus (2.8 mg/dl, ISIS range 5.9 ± 1.8) levels. Bile acids levels were 1mcmol/L (considered normal, based on the range for domestic dogs of 0.1-15 mcmol/L). At the time of the third evaluation, RA latex test, antinuclear antibody and direct Coombs were negative/normal.

Accentuated stiffness and hunched back posturing were episodically noted over the next 4.5 years, leading to immobility and requirement for hand feeding. Six years after initial presentation, she was noted to have dull, rough pelage, reduced muscle mass and palpable osseous thickening. Radiographs revealed diffuse periosteal reaction of long bones, originating at distal diaphyses (Figures 1,2). Laboratory evaluation revealed negative/normal CBC, calcium, parathormone, 25-hydroxy vitamin D, thyroid tests, BUN, creatinine, liver function test, electrocardiogram, cardiac ultrasound, treponemal rapid plasma reagin card test and fluorescent treponemal antibody-absorption test. Abnormalities in alkaline phosphatase (ALP) were detected and were characterized as fluctuating, but progressively increasing values, ranging from 663- 2200 IU/L (ISIS range 410 ± 233 IU/L).

Naproxen (62.5 mg bid) and flurbiprofen (12.5 mg tid) were without benefit. Meclofenamic acid (0.5g-1 mg/kg) and a short course of prednisone (1mg/kg) provided minimal benefit. Progressive worsening in symptoms and peripheral skeleton bony proliferation over the ensuing 18 months and brow rim prominence were accompanied by maxillary and mandibular gingival proliferation and reduction of ankle range of motion by 50%. Treatment with bicillin (50,000 IU procaine penicillin and 50,000 IU benzathine penicillin doses intramuscularly), for possibility of yaws, resulted in no benefit. Ten months later, hind limb weakness, tremor and reluctance to move were noted.

Sulfasalazine (90 mg bid) treatment resulted in increased mobility and activity, but did not halt radiologic progression. In addition, rounded caps had developed on the dorsal spinal processes of 6 lumbar vertebrae and brow ridges became more prominent (Figures 3,4). Severe stiffness and immobility subsequent to 8.25 additional years of followup led to euthanasia.

Significant gross necropsy findings were limited to the skeletal system. Severe bony proliferation was palpated on all long bones, the mandible, maxilla, and brow ridge as well as a prominent ridge of bony proliferation was present on gingival dental margin. Histopathology revealed bony proliferation (hyperostosis) characterized by bony trabeculae with an increased number of disorganized, irregularly shaped and contoured cement lines. Mild emphysema, mild splenic hemosiderosis and moderate small intestine hemosiderosis were also identified.

Case 2

A 9 year old, 3.13 kg, female red ruffed lemur (lemur 2, offspring of propositus) (100290) was anesthetized for examination due to recent episodes of stiffness and to screen

for skeletal changes as seen in its dam (lemur 1, propositus). Physical examination was unremarkable. CBC and metabolic panel were unremarkable, with exception of alkaline phosphatase of 678. Radiographs revealed significant proliferative changes in both tibia and severe proliferative changes in both radii and ulnas.

Episodes of stiffness and/or lamness occurred with increased severity occurred over the next 6.5 years with progressive increasing ALP to >1989 During this time period various treatments (ibuprofen, acetaminophen, sulfasalzine, cosequin, tramadol) were prescribed to address discomfort with variable benefit. Limited progression of radiographic lesions was appreciated in followup films. At age 16.5 years the lemur was euthanized due to non-responsive diarrhea. Necropsy information was not available.

Case 3

An 9 year old, 2.83 kg, female red ruffed lemur (lemur 3, offspring of propositus) was anesthetized for routine examination and to screen for the skeletal disease detected in its dam (lemur 1) and sibling (lemur 2). Physical examination was unremarkable. Radiographs revealed periosteal proliferation in both tibia and distal right radius. CBC and SBA were normal, with exception of phosphorus 9.6 mg/ml.

Three months later, the lemur was reluctant to move and hindlimb trembling was noted. When the animal did ambulate, she appeared to be favoring the right foot. Physical examination was unremarkable and radiographs revealed no progression of the periosteal reaction. CBC and metabolic panel were unremarkable. CPK was 34,547. Radiographs revealed early periosteal reaction.

Eight months later the animal was noted to be thin with prominent muscle wasting. Firm thickening was palpable along the tibia and ulna bilaterally. Radiographs

demonstrated a progression of lesions with hyperosotosis present along the caudolateral aspect of the proximal 1/3 of both ulna and along the cranial aspect of the proximal 1/2 of both tibiae. Normal ALP (463) and elevated BUN (46) were noted.

Episodes of stiffness and/or lamness occurred over the next 6.5 years with increased severity noted at age 16 yrs. Starting at age 11, ALP progressively increased to 1786. During this time period various treatments (ketoprofen sulfasalzine, chondroitin/glucosamine) were prescribed to address discomfort, with variable benefit. Limited progression of radiographic lesions was present in followup films, although increase brow prominence did develop similar to propositus. At age 17 years, the lemur was euthanized due to marked increase in discomfort and difficulty in moving or righting itself. Necropsy information was not available.

DISCUSSION

Diagnosis

The ruffed lemurs clearly had hypertrophic osteoarthropathy (HOA). They had generalized long bone periosteal reaction. Distal diaphyses were invariably affected, as has been documented in human HOA.[19] Hypertrophic osteoarthropathy is characterized in humans by a widespread pattern of periosteal reaction.[12-19] Although HOA is traditionally described as a disorder of distal diaphyses, more extensive disease is also noted. Periosteal reaction is found to be equally divided between distal and diffuse apposition.[19] Localization or diffuseness of periosteal reaction in the tibia does not correlate with severity of disease, extent of skeletal involvement, or which bone groups are affected in humans. The average number of affected bone groups in humans is 3.9. [Involvement of one or both tibia(e) are considered as a single bone group].

Underlying causes of secondary hypertrophic osteoarthropathy include pulmonary, gastrointestinal/esophageal, cardiac, or hepatic disease (usually abdominal or thoracic masses or lesions) are present.[12-18] None of the lemurs described in this report had radiographic evidence of a visceral neoplastic condition, nor was there evidence of non-skeletal disease based on serum liver function analysis. Antemortem cardiac evaluation (EKG and ultrasound) of the propositus, as well as gross and histological evaluation failed to show evidence or neoplasia or visceral disease.

The diagnosis of primary hypertrophic osteoarthropathy (pHOA), also called pachydermoperiostosis,[2-5,12] was made when unique facial features were recognized in the propositus and her two daughters. Furrowing/thickening of facial features was noted (Figures 3,4), often referred to as pachydrmia and cutis vertices gyrata in humans.[6] Based on the above evidence/diagnostic results, the lemurs were considered to have PHOA. Pachydermoperiostosis is a rare inherited disease that accounts for 3-5% of all cases of HOA in humans.[7] The disease exists in three forms: complete (periostosis and pachydermia), incomplete (without pachydermia), and the forme fruste (pachydermia with minimal skeletal changes). As the name implies, it is usually recognized when cutaneous lesions such a pachydermia, seborrhea, (skin changes causing coarse facial features with thickening, furrowing and excessive oiliness of the skin of face forehead, leading to acromegalic-like faces in adults), digital clubbing**,** and rarely cutis vertices gyrata occur.[4] Bone and joint involvement includes arthralgia, arthritis, periosteal new bone formation , subperiosteal ossification and osteoporosis.[4.5.7.14] Gastric mucosa hypertrophy, gastric ulcer and other endocrine abnormalities have been described.[22] PHOA may take up to 20 years for full development of symptoms in humans.[4]

Pachydermoperiostosis demonstrates an autosomal dominant mode of inheritance with variable penetrance in humans and a predilection for males predominance (over females) (7:1). Symptoms usually appear around puberty, with males generally more severely affected.[1,4,7] All lemurs affected in this report were females. It is of interest that the daughters of the propositus became affected at roughly the same age, while their male same age siblings remained disease free.

The pathogenic mechanism of PHOA is unknown. In addition to genetic influences, proposed etiological factors include anomalies in fibroblast activity or alteration of peripheral blood flow.[1,4] Support for the latter includes tortuous capillaries (as seen on nail bed capillaroscopy, capillarorhexis, microhematomas) and diffuse plasmatic and red blood cell extravasation.[5]

Differential diagnosis

Proliferative bone diseases are uncommon in nonhuman primates.[8] Prior to this report, the only bone disease with a solely adult onset that has been thoroughly described in lemurs is a familial condition of periarticular hyperostosis, seen in black lemurs (*Eulemur macaco macaco*)[10] and in black and white lemurs (*Varecia variegatatus varigatus*).[11] This contrasts with the disease observed in red ruffed lemurs, which was not limited to the periarticular region. They had generalized periosteal reaction, but no the facial bone proliferation of the form of craniodiaphyseal dysplasia referred to as lioniasis or lionitis.[23]

Differential diagnosis must consider infectious disease (e.g., treponemal), endocrinopathies with or without nutritional imbalances (e.g., thyroid acropachy, acromegaly and renal osteodystrophy or secondary hyperparathyroidism and

osteomalacia), neoplasia and hypertrophic osteoarthropathy.[14-18] Although infection of bones or surrounding tissues can cause pronounced periosteal proliferation, no evidence of an infectious process was seen in the CBC, radiographs or bone biopsies. In humans, infection with *Treponema pallidum* subsp. *pertenue*, the causative agent of yaws, a non-venereal treponematosis, can cause marked periostitis.[14,24] The possibility of treponemal infection was investigated in the propositus, but serological testing revealed no evidence of disease. As the tests have not been validated for non-human primates, a clinical treatment trial for possible yaws was initiated. It had no effect and post-mortem evaluation revealed no evidence of treponemal or other infectious agents.

Several endrocrinopathies were investigated. Thyroid acropachy causes a characteristic overgrowth of bone, probably related to overproduction of a long acting thyroid-stimulating hormone. It is easily ruled out, as it is disorder of the distal extremities, predominantly affected hands and feet,[14,18,25] areas that were not affected in the red ruffed lemurs. Similarly, acromegaly can be ruled out on the basis of absence of abnormalities of the distal portion of the extremities and of pituitary changes.[18] Although primary hyperparathyroidism has not been reported in prosiminans,[8] secondary hyperparathyroidism has been reported in black lemurs[10] and was initially considered the possible diagnosis. Hyperparathyroidism of any form (primary or secondary) was ruled out on the basis of normal calcium and absence of elevated phosphate levels and similar calcium and parathormone levels in affected and unaffected lemurs. Further evidence against secondary hyperparathyroidism includes lack of azotemia or osteomalacia and indistinguishable levels of 25-hydroxy vitamin D in affected and unaffected lemurs.

Paget's disease alters the size and shape of bones and producesa characteristic resorption pattern, in addition to the histologic findings of woven bone, cement lines, marrow fibrosis and increased marrow vascularity.[14,18] Cement lines and woven bone are non-specific, in the absence of the above-mentioned macroscopic/radiologic findings. Weber et al.[9] suggested possibility of Paget's disease in lemurs, because histology revealed prominent disorganized cement lines. However, such changes are non-specific and Paget's disease does not cause periosteal reaction. Thus, Paget's disease need not be further considered in the differential diagnosis of the ruffed lemur pathology.

Pathophysiology of disease

Variable serum ALP in affected ruffed lemurs appeared to correlate with disease activity. Bone alkaline phosphatase is a product of osteoblasts. The osteoproliferative process in the propositus may have been identified near the end of an active phase, explaining the subsequent decrease in measured ALP.

What is the significance of CPK in affected lemurs. Most values were within normal range, although phenomenal elevation was noted in one individual (CPK = 34,547). Myocardial infarction has not been previously recognized in lemurs.[8] Myocarditis was a consideration but, if present, has no known association with HOA and was not found at autopsy..

Treatment

Conventional therapy for PHOA in humans is anecdotal.[1,7] It is unclear that any have actually proven of reproducible benefit. Analgesics, NSAIDS, colchicine, and oral or intraarticular steroids have been used with minimal benefit. This contrasts with the effectiveness of sulfasalazine in treatment of the unrelated disorder, spondyloarthropathy.[26]

More recently, bisphosphonates (e.g., pamidronate, administered intravenously) have been tried, with mixed results.[7] Future cases of PHOA in lemurs may benefit from intravenous pamindronate (a biphosphonate), although minimal benefit was noted when applied herein.

CONCLUSIONS

The proliferative bone disease seen in these three ruffed lemurs appears to be a unique condition not previously described in nonhuman primates. The only bone disease with an adult onset that has been described in lemurs is a familial condition, renal osteodystrophy, seen in black (*Eulemur macaco macaco*) and in black and white lemurs (*Varecia variegatus varigatus*). The initial workup of the animals in this report did not lead to a definitive diagnosis. However, the presence of proliferative bone disease and elevated alkaline phosphatase with no other apparent metabolic derangements is most similar to PHOA as seen in humans. The apparent familial nature of this phenomenon in red ruffed lemurs is analogous to the limited (lower extremity in absence of digital clubbing) form reported in 3 members of a human family.[21]

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Figure Legends

Figure 1: Posterior-anterior x-ray view of forearms. Note massive new periosteal bone formation.

Figure 2: Oblique x-ray view of tibiae and fibulae. Note new periosteal bone formation.

Figure 3: Anterior view of facial features. A. Upper left - normal sibling of affected daughters. B. Upper right – propositus. C. Lower left - first daughter. D. Lower right - second daughter.

Figure 4: Later view of facial features. A. Upper left - normal sibling of affected daughters. B. Upper right – propositus. C. Lower left - first daughter. D. Lower right - second daughter.