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## Nephrolithiasis and Pyelonephritis in Two West Indian Manatees (*Trichechus manatus* spp.)

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ABSTRACT: Two West Indian manatees (Trichechus manatus spp.) were reported with severe emaciation. One animal was a Florida manatee from the Everglades; the other was an Antillean manatee from Cuba. On necropsy, both animals had nephrolithiasis, pyelonephritis, and moderate to severe renomegaly. Histopathology revealed multifocal to diffuse pyelonephritis, interstitial nephritis, and nephrocalcinosis. The stones were analyzed and consisted primarily of calcium carbonate. Serum chemistry values for the Florida animal revealed no renal abnormalities. The mechanism of calculus formation remains unclear in manatees. In horses, another hindgut fermenter, the most common urolith is also calcium carbonate. Urinalyses performed on manatees are very similar to those of horses (i.e., alkaline urine, low specific gravity, and calcium carbonate crystals). Formation of uroliths in manatees may have a pathogenesis similar to equine urolithiasis.

*Key words:* Kidney stone, manatee, neph-rolithiasis, pyelonephritis, renal calculi, *Trichechus manatus*.

The West Indian manatee (*Trichechus manatus* spp.) occurs in both freshwater and coastal marine habitats in Florida, often venturing west along the Gulf coast and north along the Atlantic coast. It is also found in the Greater Antilles, eastern Mexico, as well as Central and South America (Lefebvre et al., 2000). This species is divided into two subspecies, the Florida manatee (*Trichechus manatus latirostris*), occupying the US habitats, and the Antillean manatee (*Trichechus manatus manatus*), occupying the remaining regions. We report two separate cases of nephrolithiasis and pyelonephritis in the West Indian manatee, one occurring in a Florida manatee, and the other in an Antillean manatee from Cuba.

The first case, SWFTm0430b, was an adult 306-cm-long, 443.5-kg female manatee from the Florida Everglades. She was lethargic, emaciated, and had a significant list in the water. The animal was captured and transported to a rehabilitation facility for treatment, where she was orally intubated and given 21 of fresh water. Initial blood values indicated elevated creatine kinase (CK); hypoglycemia; electrolyte abnormalities including hypernatremia, hypokalemia, and hyperchloridemia; and a low iron concentration (see Table 1). Although these electrolyte abnormalities were mostly resolved following fluid administration, the manatee died several days later. At necropsy, the right kidney (25 cm  $\times$  18 cm  $\times$  7 cm, Fig. 1) was almost four times the size of the left kidney (18.5 cm  $\times$  11 cm  $\times$  4 cm). Based on data collected at the marine mammal pathobiology laboratory, the left kidney was of normal size for a manatee of this body length. The right kidney contained a  $2.5 \times 4.0$ -cm lesion in the caudal pole with a gritty necrotic center. The left kidney had a  $2.5 \times 3.5$ -cm lesion in the caudal pole that contained two large calculi, each measuring 2.0 cm in maximum diameter. The left renal pelvis was contracted and fibrotic. Histopathology of the right kidney revealed chronic active multifocal pyelonephritis, interstitial nephritis, and glomerulonephritis with

TABLE 1. Serum chemistry abnormalities of the Florida manatee SWFTm0430b before and after fluid administration.

| Parameter (recognized manatee<br>reference range [Bossart et al., 2001])   | $\underset{1^{a}}{\text{Sample}}$       | $\mathop{\rm Sample}_{2^{\rm b}}$      |
|--|---|--|
| Glucose (mg/dl) (56–117)<br>Creatine kinase (U/l) (79–302)<br>Sodium (mEq/l) (142–157)<br>Potassium (mEq/l) (4.2–6.6)<br>Chloride (mEq/l) (90–103)<br>Iron (mcg/dl) (50–199) | 33<br>11,059<br>175<br>2.8<br>134<br>12 | $78 \\ 486 \\ 147 \\ 4.5 \\ 94 \\ 156$ |

<sup>a</sup> Sample taken on admission to hospital.

<sup>b</sup> Sample taken 12 hr later.

marked cortical atrophy. The left kidney had similar lesions except that the lesions were diffuse rather than multifocal. Culture of the left kidney grew predominantly *Edwardsiella tarda* (65%) with remaining growth consisting of a nonhemolytic streptococcus species. Culture of the right kidney grew *E. tarda* (20%), *Aeromonas* sobria (20%),  $\alpha$ -hemolytic Streptococcus (20%), and miscellaneous Clostridium species. Culture of the right kidney urolith yielded *E. tarda*, *A. sobria*, and a nonhemolytic streptococcus. The urolith was analyzed and its surface layer consisted of 75% calcium carbonate and 25% calcium phosphate (apatite). The core of the stone consisted of 100% calcium carbonate.

The second case was a female 350-kg Antillean manatee from Laguna del Tesoro in the Ciénaga de Zapata, Cuba. Local residents reported a lethargic, listless manatee with extreme emaciation. A local biologist observed the manatee

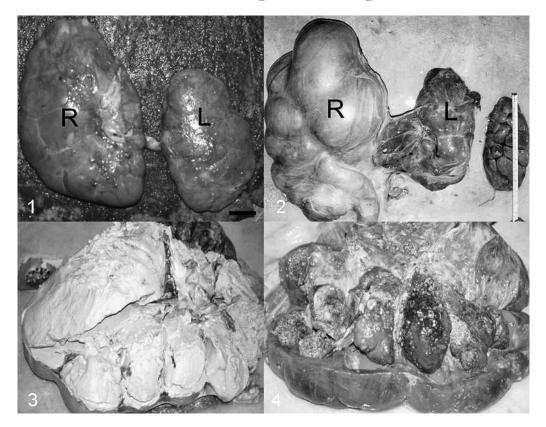


FIGURE 1. The two kidneys in the Florida manatee demonstrating renomegaly in the right kidney (R). Bar=3 cm. FIGURE 2. Right kidney of the Cuban manatee (left) compared with the left kidney (center) and to that of an adult cow kidney (right), demonstrating the renomegaly present. FIGURE 3. Sectioned kidney of the Cuban manatee showing profuse white purulent exudate. FIGURE 4. Kidney of the Cuban manatee with purulent exudate removed revealing numerous nephroliths in the pelvis of the kidney.

alive immediately prior to the arrival of the rescue team; however, the manatee died before the rescue team arrived. A field necropsy was performed but no morphometrics were recorded. Both kidneys and associated ureters were grossly enlarged and there was severe nephropathy (Fig. 2). Nodules of various sizes were present throughout both kidneys. The right kidney measured 50  $\times$  30 cm, and weighed 15 kg. On the cut surface, the right kidney had a large amount of pasty, purulent, white material along with an abundance of large and small nephroliths in the pelvis (Figs. 3 and 4). The left kidney measured  $28 \times 18$  cm and had multiple abscesses throughout the parenchyma. The kidney capsule was fibrotic and had many superficial irregularities. Histopathology revealed diffuse glomerulosclerosis and nephrocalcinosis, with areas of abscessation. Actinomyces renale was cultured and determined to be the causative agent of the suppurative process in the kidneys.

There are several reports that describe calculi in other marine mammals (Harms et al., 2004; McFee and Carl, 2004; Dennison et al., 2007). To the authors' knowledge, these are the first reported cases of nephrolithiasis in *T. manatus*. Both of these animals were severely emaciated and lethargic. Despite extensive kidney pathology, all kidney enzyme values were within normal limits in the Florida manatee. Although extensive bone remodeling and calcification of the tissues has been seen in several other manatees, neither was present in either case (Rommel, pers. comm.).

Kidney disease is not frequently seen in manatees (Bossart et al., 2004). In a search of necropsy records of the manatee necropsy database at the Florida Fish and Wildlife Conservation Commission over the last 25 yr, only 11 manatees had grossly visible primary kidney pathology. It must be noted, however, that a large portion (approximately 50%) of the manatees in the database was in an advanced state of decomposition that would preclude recognition of some lesions. Recently, there was a report of a Florida manatee with what appears to be the first case of polycystic kidney disease (Ginn, pers. comm.).

The kidneys of *T. manatus* are lobulated like those of some terrestrial mammals, but not to the extent of other marine mammals such as the Cetacea or Pinnipedia (Maluf, 1989). Because of their endangered status, studies of manatee renal function are limited. Several inferences have been made based on gross and microscopic studies of the manatee kidney. Manatees have a high medullary to cortical ratio (6:1) compared to terrestrial species, which suggests an increased ability to concentrate urine (Hill and Reynolds, 1989; Maluf, 1989). There are also several other anatomic distinctions in the manatee kidney that suggest an increased ability to concentrate urine. These include larger juxtamedullary glomeruli than cortical glomeruli and a low ratio of cortical to juxtamedullary glomerular density (Hill and Reynolds, 1989). In addition, manatees possesses thin loops of Henle that are closely juxtaposed to the vasa recta, which may increase the surface-to-volume ratio and, therefore, facilitate a countercurrent system to allow for enhancement of urine concentration (Hill and Reynolds, 1989). Manatees appear to be very good at osmoregulation independent of whether they are in fresh, brackish, or marine waters (Ortiz et al., 1998).

In manatees, the relationship of BUN and creatinine values to kidney pathology remains unclear. In one study, the blood and urine parameters were monitored in two captive manatees under simulated release conditions to determine the role stress, salinity change, and diet change have on the renal values of these animals (Manire et al., 2003). In the two animals studied, levels of serum creatinine increased as their food rations decreased. Therefore, creatinine levels in the manatee may not necessarily be the best measure of renal function. The BUN levels changed very little with diet change or a change in salinity. It did appear that during the decrease in food rations, the animals were becoming dehydrated, based on increases in total protein, red blood cell count, and hematocrit. The dehydration suggested prerenal causes for the creatinine increase and may indicate that manatees obtain much of their water through the food that they eat. However, other studies (Ortiz et al., 1999) demonstrated dehydration in saltwater animals that were deprived of fresh water over a long term period, indicating that manatees require regular access to fresh water.

The mechanism of formation of the calcium carbonate stones remains unclear. In other species, it is thought that formation occurs due to precipitation surrounding an organic nidus, such as proteins, cellular debris, crystals, or foreign bodies (Confer and Panciera, 1995). The formation of stones in these manatee kidneys could have occurred due to a preexisting pyelonephritis or other renal injury. In horses, another hindgut fermenter, the most common nephrolith reported is also calcium carbonate, and the pathogenesis still remains unclear (Mair and Osborn, 1986; Laverty et al., 1992). Typically, horses excrete calcium carbonate in their urine and it is thought to be an important part of equine calcium homeostasis (Mair, 1990). Urinalyses performed on two wild-caught manatees appear very similar to those of horses (i.e., alkaline urine, a low specific gravity, and calcium carbonate crystals [Harr, pers. comm.]). This was also seen during experiments performed on captive manatees (Manire et al., 2003) Formation of uroliths in manatees may have a pathogenesis similar to equine urolithiasis.

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