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# Struvite Calculus in the Vagina of a Bottlenose Dolphin (*Tursiops truncatus*)

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ABSTRACT: On 27 January 2000, a struvite calculus was observed in the vagina during necropsy of a 138-cm-long female bottlenose dolphin (*Tursiops truncatus*) collected from the Stono River, South Carolina (USA). Vaginal calculi have been reported in other species of cetaceans but not in bottlenose dolphins. Urinary tract infection might have been an underlying cause of the calculus. While urinary tract inflammation was not detected by light microscopic evaluation of sections of the urinary tract, it is conceivable that sufficient time had lapsed following voiding of the calculus through the urethra for urinary tract infection to have resolved. To further define the prevalence and significance of urolithiasis, prosectors of dead stranded marine mammals are encouraged to closely observe their urinary and genital tracts for calculi and to submit them for quantitative analysis.

*Key words:* Bottlenose dolphins, case report, struvite, *Tursiops truncatus,* vaginal calculus.

Vaginal calculi have not been described in bottlenose dolphins (*Tursiops truncatus*), but they have been described in common dolphins (*Delphinus delphis*) (Harrison, 1969; Sawyer and Walker, 1977; Benirschke et al., 1984; Woodhouse and Rennie, 1991), northern right whale dolphin (*Lissodelphis borealis*) (Woodhouse and Rennie, 1991), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Sawyer and Walker, 1977), spotted dolphins (*Stenella attenuata*) (Sawyer and Walker, 1977), and Peruvian dusky dolphins (*Lagenorhynchus obscurus*) (Van Bressem et al., 2000). These calculi have been attributed to vaginal plugs formed from coagulated seminal fluid (Harrison, 1969), fetal bone remnants (Sawyer and Walker, 1977; Benirschke et al., 1984; Woodhouse and Rennie, 1991), and bacterial infections (Van Bressem et al., 2000). Here we describe a vaginal calculus composed primarily of struvite (magnesium ammonium phosphate hexahydrate) in a bottlenose dolphin.

On 27 January 2000, a 138-cm-long female bottlenose dolphin was found dead on the bank of the Stono River, Charleston County, South Carolina (USA, 32°43.13N, 79°59.22W). The animal was transported to the National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina for necropsy. Gross abnormalities detected at necropsy included lordoscoliosis, 0.1 mm dot-like skin lesions, severe ankylosing osteoarthritis of the vertebral joints and atlanto-occipital joint, necrotic epaxial muscle, a few renal calculi, a 13.0-×6.0cm circular lesion ventral to the dorsal fin invading the blubber layer and surrounding fascia, and a 4.0cm-diameter 52.3g vaginal calculus with associated infection (Fig. 1). The animal was aged at 4 yr using standard tooth thin-section techniques (Hohn et al., 1989). This age was verified from a photo-identification catalogue held at CCEHBR. The animal was first sighted with lordoscoliosis in the Stono River on 24 January 1997 and resightings occurred six times from March 1997 to January 2000. The last sighting of this animal alive was on 21 January 2000, 6 days prior to discovery of the animal dead on the Stono River bank (E. Zolman, pers. comm.).

Formalin-fixed tissues (Accession number 2716501 00) were sent to the Armed Forces Institute of Pathology (Washington, D.C., USA). Histopathology revealed blubber lesions characterized by an inflammatory response associated with crys-

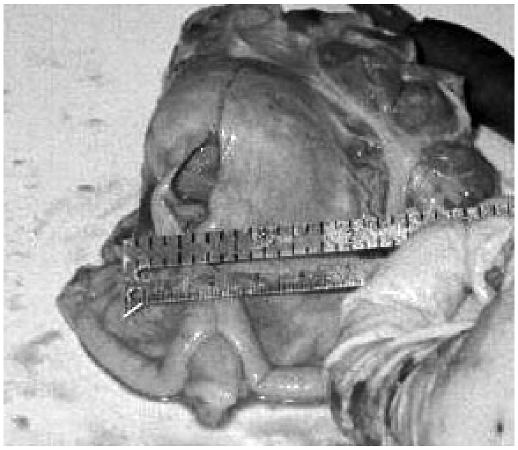

FIGURE 1. Calculus in the vagina of a bottlenose dolphin.

talline structures that resembled urate crystals seen in gout. The only case of gout in cetaceans found in the literature was cutaneous gout in a captive Amazon dolphin (*Inia geoffrensis*) (Garmen et al., 1983). No microscopic lesions were found in the kidneys and urinary bladder. Mild vaginitis, cervicitis, hepatitis, pancreatitis, and enteritis interpreted to be clinically insignificant were also observed.

The vaginal calculus (Accession number 159368DLS) was sent to the Minnesota Urolith Center at the University of Minnesota, College of Veterinary Medicine (St. Paul, Minnesota, USA) for quantitative analysis of its mineral composition by polarizing light microscopy (Ulrich et al., 1996). The majority of the calculus consisted of magnesium ammonium phosphate hexahydrate (struvite). Two bands were detected within the calculus. One band was composed of 85% struvite and 15% ammonium urate and the other band was composed of 95% struvite and 5% ammonium urate.

Female bottlenose dolphins attain sexual maturity between 5 and 10 yr of age (Reynolds et al., 2000), indicating that the dolphin described here was unlikely to have had sexual contact and corpora albicantia were not found on the ovaries of this bottlenose dolphin. The composition of struvite and ammonium urate and the sexually immature status of this bottlenose dolphin suggests that formation of the vaginal calculus was due to an etiology other than fetal bone remnants or seminal fluid plugs as suggested by previous authors. A more plausible explanation is that the vaginal calculus was the result of an infection possibly originating from the urinary tract similar to the pathogenesis suggested by Van Bressem et al. (2000).

For struvite calculi to form, urine must be supersaturated with magnesium ammonium phosphate hexahydrate. However, acidic urine of healthy dolphins is likely to be undersaturated with magnesium, ammonium, and phosphate. Normally, urine ammonium ($NH_4^+$) concentration increases only when acid catabolites are excreted in high concentrations by the kidneys. The increase in urine concentration of ammonium in this situation represents a normal compensatory response by the renal tubular cells to secrete ammonia ($NH_3$) into the tubular lumen to reduce acidity by subsequent formation of ammonium ($NH_4^+$). Whereas ammonia is lipid soluble and can penetrate tubular cell walls, ammonium is lipid insoluble and cannot penetrate cell walls (so-called ion trapping). Likewise, excretion of alkaline urine under physiologic conditions is associated with reduced renal production of ammonia and, thus, reduced quantities of ammonium ions in urine.

When urinary tract infection with urease-producing microbes occurs in animals forming urine with a sufficient quantity of urea, increase in the concentration of ammonium in an alkaline environment develops, favoring formation of struvite calculi. The following mechanisms are involved (Osborne et al., 1985). Urease produced by bacteria or ureaplasmas hydrolyze urea to form two molecules of ammonia and a molecule of carbon dioxide. The ammonia molecule reacts spontaneously with water to form ammonium and hydroxyl ions, which in turn alkalinizes urine by reducing hydrogen ion concentration. The solubility of struvite decreases in alkaline urine. In addition to alkalinizing urine, the newly generated ammonium ions are available for formation of magnesium ammonium phosphate crystals. In the progressively alkaline environment induced by microbial hydrolysis of urea, dissociation of monobasic hydrogen phosphate ($H_2PO_4^{1-}$) results in an increased concentration of dibasic hydrogen phosphate ($HPO_4^{2-}$) and anionic phosphate ($PO_4^{3-}$). Anionic phosphate is then available in increased quantities to combine with magnesium and ammonium to form struvite. Ammonium ions may also combine with uric acid to form ammonium urate crystals.

A sufficient quantity of urea and urease are required for ammonia production, alkalinization, supersaturation, and subsequent precipitation of struvite crystals. The majority of urea in urine originates from dietary protein, whereas urease in vertebrates must be derived from microbes (some bacteria, ureaplasma, and some yeasts). The high concentration of urea present in urine of individuals that consume dietary protein in excess of daily requirements for protein metabolism makes urine a suitable environment to support the pathogenic effects of urease-producing microbes.

In light of the pathophysiology of formation of infection-induced struvite calculi, it is unlikely that the calculus found in this dolphin originated in the vagina. The only plausible alternative is that it originated in the urinary bladder and was voided into the vagina through the urethra. However, if the calculus composed of infection-induced struvite was voided through the urethra and trapped in the vaginal lumen, one would expect to find evidence of urinary-tract infection. Gross observations of infection surrounding the uterine tissue and in the blubber and musculature around the genital opening would support this suggestion of a urinary infection. In humans, vaginal calculi are rare and believed to originate from the stasis of urine without a nidus or the result of urine crystallization around a foreign body in the vagina (Plaire et al., 2000). Plaire et al. (2000) also suggest that adult women develop vaginal calculi from a vesicovaginal fistula and concomitant urinary tract infection, but in children, reported predisposing factors include an ectopic ureter, a neurogenic bladder with incontinence, and partial vaginal outlet obstruction. In this bottlenose dolphin, urinary tract inflammation was not detected by light microscopic evaluation of sections of the urinary tract. It is conceivable that sufficient time had lapsed following voiding of the calculus through the urethra for urinary tract infection to have resolved.

Woodhouse and Rennie (1991) observed heavy infection of the cestode *Monorygma* sp. in the reproductive tract of a common dolphin associated with a vaginal calculus. The parasitic infection, therefore, may have served as a nidus for the vaginal calculus to form. The bottlenose dolphin described here had mild lymphoplasmacytic and eosinophilic inflammation of multiple organs, suggesting parasitism, but no parasites were observed in the vagina or bladder.

While vaginal calculi may be a common condition in cetaceans, this is the first report in the literature of a vaginal calculus in a bottlenose dolphin. To further define the prevalence and significance of urolithiasis, prosectors of dead stranded marine mammals are encouraged to closely observe their urinary and genital tracts for calculi and to submit them for quantitative analysis. We recommend collecting and submitting all calculi found in the urinary or genital tracts. Calculi may form at different times; therefore, their mineral composition may vary from stone to stone. If multiple calculi are present in different locations, place them in separate, clearly labeled containers. Do not fragment or crush calculi as this will distort different layers of minerals within the stone. Package calculi in crush-proof containers. Submit uroliths dry and do not place them in formalin, as this may alter the composition of some minerals. Accurately and completely fill out the appropriate laboratory submission form. Request that quantitative methods of analysis be utilized because qualitative methods of calculi analysis are unreliable.

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