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Vol. 132: 79–84, 2018
https://doi.org/10.3354/dao03305

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 11

NOTE

Aeromonas induced polyostotic osteomyelitis in a juvenile loggerhead sea turtle *Caretta caretta*

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ABSTRACT: Bacterial bone infections have been occasionally reported in wild sea turtles. This study reports on a sub-adult *Caretta caretta* affected by *Aeromonas hydrophila* osteomyelitis, with extensive involvement of cranial and caudal flippers. The turtle was severely debilitated, had limited flipper mobility and showed signs of pain in reaction to manipulation. Radiographs and computed tomography revealed multiple lytic bone lesions. Since an infectious polyostotic osteomyelitis was suspected, the turtle was subjected to echo-assisted fine needle aspiration to characterize the etiology of the disease. Bacterial cultures and antibiotic susceptibility testing led to the isolation of *Aeromonas hydrophila* responsive to amikacin and doxycycline. Therefore, the turtle was treated with these antibiotics and monitored through repeat bacterial cultures and diagnostic imaging. The turtle was released 17 mo after admission, upon resolution of clinical signs. The documentation of this case provides a treatment approach that may improve the outcome of *Aeromonas*-associated osteomyelitis, especially in endangered wildlife species.

KEY WORDS: Polyostotic osteomyelitis · *Caretta caretta* · *Aeromonas hydrophila* · Bacterial bone infection

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1. INTRODUCTION

Bacterial infections can occur in both free-ranging and captive sea turtles, mainly caused by opportunistic pathogens, leading to stomatitis, dermatitis, pneumonia and bone infections (Innis & Frasca 2017). Localized or generalized inflammation and destruction of bones is classified as osteomyelitis. The disease can progress to local inflammation and stiffening of soft tissues, reduced motility, osteolysis, septicaemia, and death (Fitzgerald & Vera 2006). In reptiles, osteomyelitis is characterized by both slow progressive lytic processes, predominantly in the appendicular skeleton, and a proliferative response, which is particularly expressed in the vertebrae of snakes and lizards (Silverman 2006, Di Girolamo et al. 2014). Local anatomy is often altered by bone remodelling in response to infection, and a persistent lytic defect is often seen after resolution of the process (Silverman 2006). Diagnosis is based on a combination of supporting clinical findings and laboratory investigations, including cytology/biopsy, microbial cultures and special stains. In addition, radiography, computed tomography (CT) and scintigraphy are valuable imaging techniques to investigate skeletal abnormalities (Fitzgerald & Vera 2006, Solano et al. 2008).

Aeromonas species are facultatively anaerobic, Gram-negative rods that are ubiquitous microorganisms, and which are globally found in a broad range

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of habitats, especially aquatic environments (Igbinosa et al. 2012). Additionally, *Aeromonas* have been isolated from food samples (Buchanan & Palumbo 1985, Krovacek et al. 1994, Janda & Abbott 2010). They have been considered as normal gastrointestinal flora of fish and reptiles and are sometimes found in the intestinal tract of humans and livestock (Janda & Abbott 2010, Igbinosa et al. 2012). In these hosts, *Aeromonas* show different virulence and pathogenicity, mostly resulting in opportunistic diseases. In particular, fish, amphibians and reptiles are the most commonly documented animals affected by *Aeromonas* infections (Igbinosa et al. 2012). The interest in the pathogenic nature of *Aeromonas* species has increased over time, because the *Aeromonas* genus is a significant threat to animal and human health alike (Janda & Abbott 2010).

The present study reports on the case of *A. hydrophila*-induced osteomyelitis with extensive involvement of cranial and caudal flippers in a sub-adult loggerhead sea turtle (*Caretta caretta*).

2. CASE PRESENTATION

2.1. Clinical findings and maintenance

A sub-adult loggerhead sea turtle (curved carapace length: 53.5 cm; body mass: 17.7 kg) was found along the south-eastern coast of Italy (Adriatic Sea), and admitted to the Marine Turtle Research Centre (MTRC) of the Stazione Zoologica Anton Dohrn of Naples on 23 December 2015. The turtle was lethargic, debilitated, dehydrated, and had no appetite, reduced flipper movements and showed signs of pain during manipulation. Since the turtle was at risk of drowning because of its reduced swimming ability, it was initially kept in shallow water and monitored for the first 24 h while slowly increasing the water level to 0.5 m. The turtle floated and was kept at this water height and at a temperature of 22°C. An esophagostomy tube had been placed at the University of Bari, where the turtle had been primarily treated after rescue for 7 wk, before being transferred to the MTRC. The tube was maintained to administer 200 g of a mash of fishmeal, until the turtle started to accept hand-fed fresh anchovies. When the turtle started feeding on its own, a daily food ration of approximately 200 g, corresponding to 1% of its body mass, was administered. Bloodwork revealed an increased white blood cell count (29.6×10^9 l^{-1}) with a heterophil to lymphocyte ratio of 0.64, suggesting a chronic inflammation. Packed cell volume was 19% and total proteins were 5.2 g dl^{-1}, within the range reported for sub-adult loggerheads (Stacy & Innis 2017).

2.2. Diagnostic imaging

Initial radiographs were taken at the University of Bari 6 wk prior to admission to the MTRC, where a left coxo-femoral luxation was diagnosed. On 4 January 2016, radiographic exams were repeated at the Interdepartmental Centre of Veterinary Radiology of the University of Naples Federico II, showing a lytic left femur head and a fracture of the right acromion (Fig. 1). The turtle continued to float, did not regain the motility of the flippers, and a longitudinal paramedial fracture appeared on the right side of the plastron, progressively extending up to 13 cm.

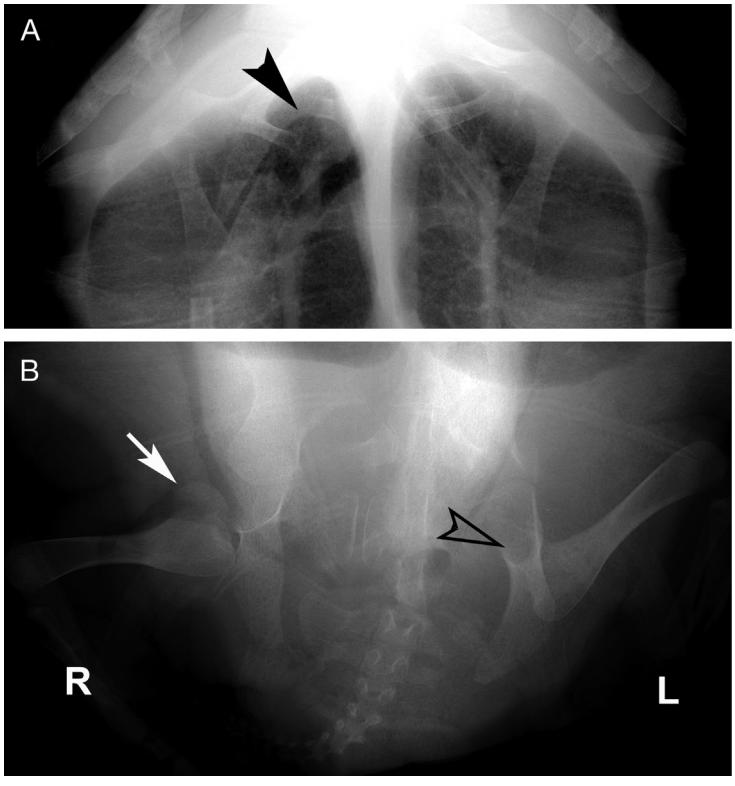

Fig. 1. Radiographs of the (A) pectoral and (B) pelvic girdle in dorso-ventral view. Note the (A) fractured right acromion process (black arrowhead), and (B) the lytic left femoral head (empty arrowhead) compared to the normal contralateral (white arrow). R: right body side; L: left body side

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Therefore on 19 February 2016 computed tomography (CT) was performed revealing additional lytic bone lesions of the left scapula, left acromion, left humerus head, right coracoid, left acetabulum, right pubis and right ischium (Fig. 2). All the bone lesions showed similar permeative lytic features, complicated by pathologic fractures at the level of the left scapula and acromion and the right acromion and ischium. Based on the radiographic and CT findings, an infectious polyostotic osteomyelitis was suspected. Therefore, an ultrasonographic exam, through a left prefemoral window, followed by an echo-assisted fine needle aspiration from the left femur head, was conducted (Fig. 3), and collected samples were used for bacterial and fungal cultures. Cytology was not performed, although it would have been ideal for a complete work-up.

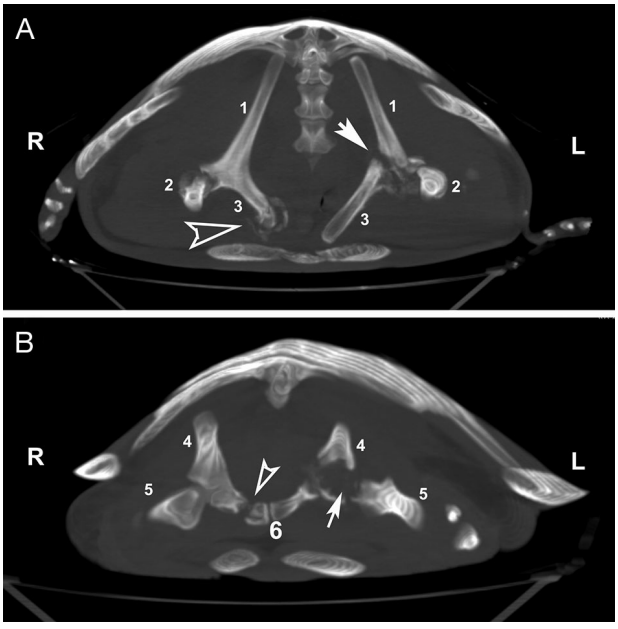

Fig. 2. Computed tomography maximum intensity projection (MIP) reconstruction of the (A) pectoral and (B) pelvic girdle. Note that in (A) there are bone lyses and a pathologic fracture with a small callus of the right acromion process (empty arrowhead), and of the insertion on the scapula of the left acromion process (white arrow). In (B) there are bone lyses of the left femoral head and acetabulum (white arrow) and a fractured right ischium (empty arrowhead). R: right body side; L: left body side; 1: scapula; 2: insertion of the coracoid to the still unfused scapula; 3: acromion process; 4: ileum; 5: femur; 6: ischium

2.3. Bacterial isolation

Three samples were obtained through needle aspiration and processed for bacterial and fungal isolation. The samples were split and enriched in buffered peptone water (Oxoid) and Alkaline saline peptone water for 18–24 h at 30°C, and subsequently plated onto different enrichment and selective agar plates. Sabouraud dextrose agar (Oxoid) plates were used for fungal isolation. Specific cultures and acid-fast stain for *Mycobacterium* spp. were not performed. Cultures on Columbia blood agar (Oxoid) plates demonstrated large (~3–4 mm diameter), white, creamy, β-haemolytic and oxidase positive colonies; whereas on MacConkey agar no. 3 (Oxoid) plates, cultures demonstrated lactose non-fermenting, oxidase positive colonies. No other microorganism was isolated from any bacterial and fungal culture. Bacteria stained as Gram-negative bacilli and were identified

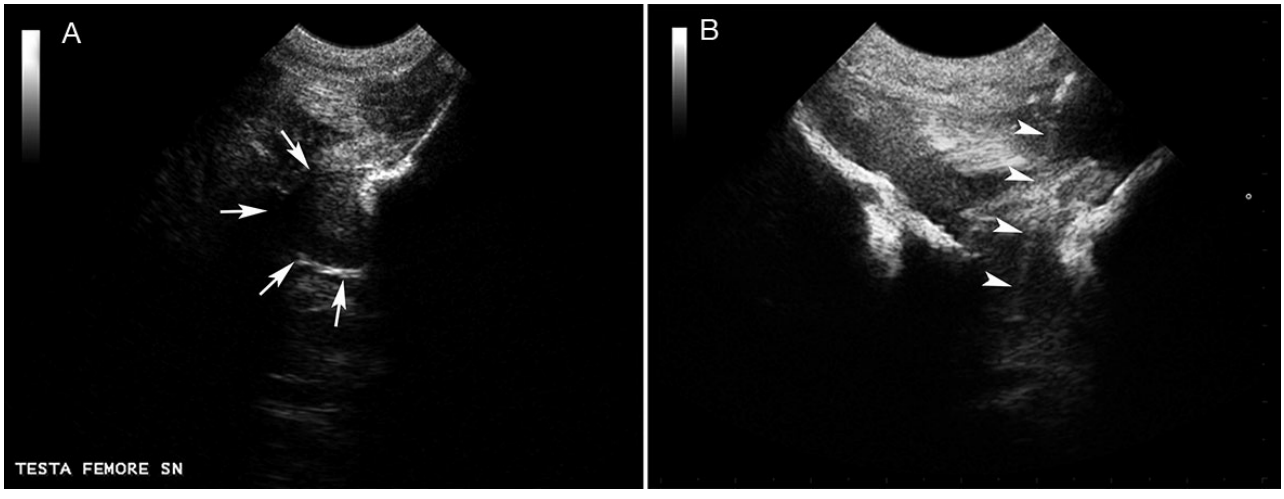

Fig. 3. Transversal ultrasonographic scan of the left coxofemoral joint. (A) The lytic femoral head is hypoechoic (white arrows). (B) The same scan obtained during the fine needle aspiration from the left femoral head with the needle inserted (arrowheads)

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as *A. hydrophila* by the BioMerieux index system. The strain was submitted to antimicrobial susceptibility testing using the disk diffusion method, according to the Clinical and Laboratory Standards Institute documents (CLSI 2012). The antimicrobials (Oxoid) tested were amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), doxycycline (30 µg) and lincomycin (2 µg). According to the interpretive criteria (Lamy et al. 2012, CLSI 2014), the *A. hydrophila* strain was found susceptible to amikacin and doxycycline, intermediately susceptible to gentamicin, and resistant to ceftazidime, ciprofloxacin and lincomycin.

2.4. Treatment

The turtle was initially treated with marbofloxacin (Aristos; 5 mg kg^{-1} subcutaneously every 48 h) in combination with ceftazidime (Glazidim; 20 mg kg^{-1} intramuscularly every 72 h) for 3 wk, but did not show any improvement. Therefore, when the results of the antimicrobial susceptibility testing became available, the treatment was changed to amikacin (Amikavet; 125 mg ml^{-1} at the dose of 5 and 2.5 mg kg^{-1} intramuscularly every 48 h) in combination with doxycycline (Ronaxan; 250 mg $tablet^{-1}$ at the dose of 25 mg kg^{-1} orally every 72 h) for 16 wk.

Other supporting therapies consisted of administrating analgesics (Tramadol; 6 mg kg^{-1} orally every 72 h for 4 wk and then under necessity) and fluid therapy 10 ml kg^{-1} (including 5 ml kg^{-1} of Ringer's solution; 2.5 ml kg^{-1} of saline solution and 2.5 ml kg^{-1} of 5% glucose solution, subcutaneously every 24 h 5 d wk^{-1}) for the first 2 mo. Then glucose was suspended and fluid therapy continued with 7.5 ml kg^{-1} 3 d wk^{-1} for 4 mo. Vitamin and mineral supplements (Stimulfoss; 0.1 ml kg^{-1} wk^{-1}; Aquavits: 1.5 tablet wk^{-1}) were also administered.

The turtle's clinical improvement was corroborated by increased appetite and weight gain (23% of the initial body mass), reaction to external stimuli, and no signs of pain during manipulation. The turtle also regained motility and buoyancy control and, hence ceased to float and spent time submerged on the bottom of the tank. Bacterial culture was repeated on 27 September 2016 and serial imaging studies were performed on 1 April and 18 May 2017. The bacterial cultures were negative, suggesting a resolution of the infection. The radiology revealed a decrease in osteolytic areas and a peripheral sclerotic response (Fig. 4). This pattern of healing was similar in all bone lesions, indicating a resolving osteomyelitis. The turtle was released on 25 May 2017, 1 yr after the end of antibiotic treatment and upon resolution of clinical signs,

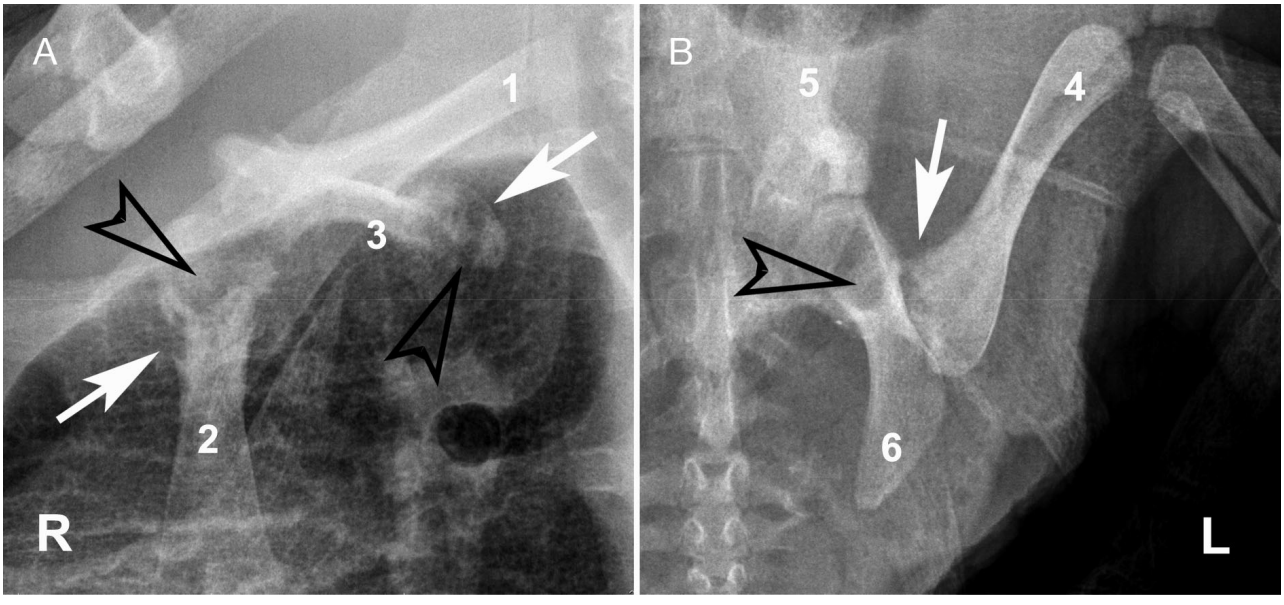

Fig. 4. Final control radiographs of the (A) right pectoral and (B) left pelvic girdle in dorso-ventral view (cf. Fig. 1). In (A) there are still visible bone lyses on the acromion process and at the level of the coracoid (empty arrowheads) but, at the same time, areas of sclerotic and periosteal proliferation surround the lesions (white arrows). In (B) the left femoral head is lytic and partially superimposed to the acetabulum (empty arrowhead); a slight periosteal proliferation and bone sclerosis are visible on the neck and proximal diaphysis of the femur (white arrow). R: right body side; L: left body side; 1: scapula; 2: coracoid; 3: acromion process; 4: femur; 5: ileum; 6: ischium

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which included a normal range of mobility of all flippers.

3. DISCUSSION

Cases of osteomyelitis documented in reptiles reported *Salmonella* spp. as one of the most commonly cultured Gram-negative bacteria. Other etiologic agents associated with this condition include *Serratia marcescens, Enterococcus* spp., *Pseudomonas* spp., *E. coli, Proteus* spp., *Mycoplasma* spp., and fungi, which were reported in crocodiles, iguanas, snakes and lizards (Jacobson 2007). Only 1 case of *Aeromonas* spp. cultured from the lesion of a tibia affected by osteomyelitis was reported in an iguana (Mader & Bennett 2006). Osteomyelitis has been documented in sea turtles, specifically in cases of cold-stunning; penetrating injuries and pneumonia; bacterial infections from *Mycobacterium chelonae*, *Vibrio alginolyticus*, *Nocardia* spp., *Serratia marcescens*, *Enterococcus faecalis* and coagulase positive *Staphylococci*; fungal infections from *Scolecobasidium constrictum* and another unidentified fungal pathogen (Ogden et al. 1981, Leong et al. 1989, Harms et al. 2002, Greer et al. 2003, Solano et al. 2008, Innis et al. 2014). *Aeromonas* spp. in sea turtles are typically found in association with other microorganims, from a variety of other lesions, such as pneumonia, nephritis, hepatitis, digestive lesions, dermatitis and from the complex ulcerative stomatitis–obstructive rhinitis–bronchopneumonia (Work et al. 2003, Orós et al. 2005).

In sea turtles the most common routes for bacterial infection are traumatic injuries and aspiration of water where bacteria can subsequently enter the bloodstream and disseminate throughout the entire body (Work et al. 2003). In this case, it is possible that the loggerhead turtle developed osteomyelitis as a consequence of *A. hydrophila* septicaemia from an initial site that could not be established with certainty. *Aeromonas hydrophila* septicaemia is a well-known threat to wild and captive reptiles, in particular those inhabiting aquatic environments (Pasquale et al. 1994, Roh et al. 2011). Despite the severity of septicaemic disease, affected animals might exhibit non-specific signs or die suddenly without clinical signs (Turutoglu et al. 2005). Whenever a septicaemia is suspected, blood culture is indicated. In our case haemoculture was not performed, because the pathogen was isolated directly from the bone lesion. Hematogenous spread of bacteria has been frequently reported as responsible for osteoarthritis and septic arthritis in sea turtles (Greer et al. 2003, Raidal et al. 2006, Guthrie et al. 2010), although in our case diagnostic imaging did not show thickening of articular capsules and synovial effusion, excluding joint involvement. However, in this case, there was likely a hematogenous spread given the multiple anatomic sites affected.

Radiography still represents the main diagnostic tool to assess skeletal disorders (Valente et al. 2007b, Solano et al. 2008). However, CT scan, providing 3D characterization of the tissue, is the imaging tool of choice to characterize bone lesions (Abou-Madi et al. 2004, Valente et al. 2007a). In the present case, we used radiography, CT and ultrasonography to achieve the diagnosis. Although radiography showed lesions on some bones, it demonstrated a lower sensitivity compared to the CT. Ultrasonography is useful to collect the sample for microbiology, as it permits real-time visualization of the biopsy site (Valente et al. 2007c).

Broad-spectrum antimicrobials and antifungal medications in cold-stunned turtles were suggested to be effective on some osteomyelitic lesions (Solano et al. 2008). Most *Aeromonas* species show *in vitro* susceptibilities to aminoglycosides, tetracyclines, chloramphenicol, sulfamethoxazole trimethoprim and quinolones, while resistances have been detected to nalidixic acid, ciprofloxacin and norfloxacin (Igbinosa et al. 2012). According to the results of antibiotic susceptibility testing, our patient was successfully treated with a combination of amikacin and doxycycline for a prolonged period of time, whereas fluoroquinolones were not effective, suggesting that these antimicrobials would not be the first option for treating *Aeromonas* infections.

Given the increasing interest in the pathogenic nature of *Aeromonas* spp. over recent years due to their disease spectrum, antimicrobial-resistance patterns, and ubiquitous presence (Krovacek et al. 1994), this bacterium should be considered in differential diagnosis of osteomyelitis in marine turtles.

Acknowledgements. Animal handling procedures were performed according to the authorization by the Ministry of Environment and Protection of Land and Sea (Protocol n.0042848/PNM 09/08/2013 and Protocol n.0024471/PNM 22/11/2016). This work was supported by a PhD fellowship of the University Federico II and the Stazione Zoologica Anton Dohrn of Naples. The authors thank the personnel of the Wildlife Rescue Centre 'Federico II' (University of Naples) and of the Veterinary Hospital (Local Health Authority) of Naples. Additionally, the authors acknowledge Prof. Antonio Di Bello (University of Bari) for his role in the primary treatment of the sea turtle.

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LITERATURE CITED

Abou-Madi N, Scrivani PV, Kollias GV, Hernandez-Divers SM (2004) Diagnosis of skeletal injuries in chelonians using computed tomography. J Zoo Wildl Med 35: 226–231

Buchanan RL, Palumbo SA (1985) *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: a review. J Food Saf 7:15–29

CLSI (Clinical and Laboratory Standards Institute) (2012) Performance standards for antimicrobial disk susceptibility tests. Approved Standard, 11th edn. CLSI document M02-A11. CLSI, Wayne, PA

CLSI (Clinical and Laboratory Standards Institute) (2014) Performance standards for antimicrobial susceptibility testing. Twenty-fourth informational supplement. CLSI document M100-S24. CLSI, Wayne, PA

Di Girolamo N, Selleri P, Nardini G, Corlazzoli D and others (2014) Computed tomography-guided bone biopsies for evaluation of proliferative vertebral lesions in two boa constrictors (*Boa constrictor imperator*). J Zoo Wildl Med 45:973–978

Fitzgerald KT, Vera R (2006) Spinal osteopathy. In: Mader DM (ed) Reptile medicine and surgery, 2nd edn. Saunders Elsevier, Philadelphia, PA, p 906–912

Greer LL, Strandberg JD, Whitaker BR (2003) *Mycobacterium chelonae* osteoarthritis in a Kemp's ridley sea turtles (*Lepidochelys kempii*). J Wildl Dis 39:736–741

Guthrie A, George J, deMaar TW (2010) Bilateral chronic shoulder infections in an adult green sea turtle (*Chelonia mydas*). J Herpetol Med Surg 20:105–108

Harms CA, Lewbart GA, Beasley J (2002) Medical management of mixed nocardial and unidentified fungal osteomyelitis in a Kemp's ridley sea turtle, *Lepidochelys kempii*. J Herpetol Med Surg 12:21–26

Igbinosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI (2012) Emerging *Aeromonas* species infections and their significance in public health. Sci World J 2012:625023

Innis CJ, Frasca S (2017) Bacterial and fungal diseases. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds) Sea turtle health and rehabilitation. JRoss Publishing, Plantation, FL, p 779–790

Innis CJ, Braverman H, Cavin JM, Ceresia ML and others (2014) Diagnosis and management of *Enterococcus* spp infections during rehabilitation of cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*): 50 cases (2006–2012). J Am Vet Med Assoc 245:315–323

Jacobson ER (2007) Bacterial diseases of reptiles. In: Jacobson ER (ed) Infectious diseases and pathology of reptiles. Color atlas and text. CRC Press, Boca Raton, FL, p 461–526

Janda JM, Abbott SL (2010) The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23:35–73

Krovacek K, Pasquale V, Baloda SB, Soprano V, Conte M, Dumontet S (1994) Comparison of putative virulence factors in *Aeromonas hydrophila* strains isolated from the marine environment and human diarrheal cases in southern Italy. Appl Environ Microbiol 60:1379–1382

Lamy B, Laurent F, Kodjo A, Roger F, Jumas-Bilak E, Marchandin H (2012) Which antibiotics and breakpoints should be used for *Aeromonas* susceptibility testing? Considerations from a comparison of agar dilution and disk diffusion methods using *Enterobacteriaceae* breakpoints. Eur J Clin Microbiol Infect Dis 31:2369–2377

Leong JK, Smith DL, Revera DB, Clary JC, Lewis DH, Scott JL, DiNuzzo AR (1989) Health care and diseases of captive-reared loggerhead and Kemp's ridley sea turtles. In: Caillouet CW Jr, Landy AM Jr (eds) Proc 1st Int Symp Kemp's Ridley Sea Turt Biol Conserv Manag. Texas A&M University, Sea Grant College Publication, Galveston, TX, p 178–201

Mader DR, Bennett RA (2006) Soft tissue, orthopedics, and fracture repair. In: Mader DM (ed) Reptile medicine and surgery, 2nd edn. Saunders Elsevier, Philadelphia, PA, p 581–612

Ogden JA, Rhodin AG, Conlogue GJ, Light TR (1981) Pathobiology of septic arthritis and contiguous osteomyelitis in a leatherback turtle (*Dermochelys coriacea*). J Wildl Dis 17:277–287

Orós J, Torrent A, Calabuig P, Déniz S (2005) Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998–2001). Dis Aquat Org 63: 13–24

Pasquale V, Baloda SB, Dumontet S, Krovacek K (1994) An outbreak of *Aeromonas hydrophila* infection in turtles (*Pseudemis scripta*). Appl Environ Microbiol 60: 1678–1680

Raidal SR, Shearer PL, Prince RIT (2006) Chronic shoulder osteoarthritis in a loggerhead turtle (*Caretta caretta*). Aust Vet J 84:231–234

Roh YS, Park H, Cho HU, Cho A and others (2011) *Aeromonas hydrophila*-associated septicemia in captive crocodiles (*Crocodylus johnstoni* and *Crocodylus porosus*). J Zoo Wildl Med 42:738–742

Silverman S (2006) Diagnostic imaging. In: Mader DM (ed) Reptile medicine and surgery, 2nd edn. Saunders Elsevier, Philadelphia, PA, p 471–489

Solano M, Innis C, Smith C, Merigo C, Weber ES III (2008) Scintigraphic and radiographic evaluation of appendicular skeletal lesions in cold-stunned Kemp's ridley sea turtles. Vet Radiol Ultrasound 49:388–394

Stacy NI, Innis CJ (2017) Clinical pathology. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds) Sea turtle health and rehabilitation. JRoss Publishing, Plantation, FL, p 147–207

Turutoglu H, Ercelik S, Corlu M (2005) *Aeromonas hydrophila*-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*): clinical communication. J S Afr Vet Assoc 76:40–42

Valente AL, Cuenca R, Zamora M, Parga ML, Lavin S, Alegre F, Marco I (2007a) Computed tomography of the vertebral column and coelomic structures in the normal loggerhead sea turtle (*Caretta caretta*). Vet J 174: 362–370

Valente AL, Marco I, Zamora MA, Parga ML, Lavín S, Alegre F, Cuenca R (2007b) Radiographic features of the limbs of juvenile and subadult loggerhead sea turtles (*Caretta caretta*). Can J Vet Res 71:305–313

Valente AL, Parga ML, Espada Y, Lavin S, Alegre F, Marco I, Cuenca R (2007c) Ultrasonographic imaging of loggerhead sea turtles (*Caretta caretta*). Vet Rec 161:226–232

Work TM, Balazs GH, Wolcott M, Morris R (2003) Bacteraemia in free-ranging Hawaiian green turtles *Chelonia mydas* with fibropapillomatosis. Dis Aquat Org 53:41–46

Editorial responsibility: Thierry Work, Honolulu, Hawaii, USA

Submitted: February 26, 2018; Accepted: October 16, 2018
Proofs received from author(s): November 16, 2018