Trunk vertebrae osteomyelitis in a spectacled caiman (*Caiman crocodilus*)

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ABSTRACT - Osteomyelitis is frequently reported in turtles, lizards and snakes and *Salmonellae* are increasingly reported as the causative organism. However, very little is known of this disease occurring in crocodilians. Crocodilians are shy, robust animals and often hide clinical symptoms, especially when submerged in water. Knowledge of disease in crocodilians is important, especially in zoos and farms where they are often kept in high densities. Here we report the first known case of trunk vertebrae osteomyelitis in the order Crocodilia. A six year old, captive raised spectacled caiman (*Caiman crocodilus*) was admitted for veterinary examination after developing a postural abnormality in the vertebral region, including spinal curvature and dorsal indentation. Radiographic and Computed Tomographic studies showed muscle wastage and multifocal vertebral osteolytic changes suggestive of osteomyelitis. The caiman was euthanized and post-mortem examination revealed coelomic serosanguinous effusion and a diffuse severe fibrinous coelomitis with firm visceral adhesions involving the liver and spleen. A 15 cm irregular mass was found within and greatly expanding a major blood vessel ventral to the trunk vertebrae. Examination of the mass revealed an organized thrombus and examination of the affected trunk vertebrae revealed severe osteolytic changes with extensive remodeling. *Salmonella enterica houtenae* was isolated from the vertebral lesions. *Salmonellae*, a common constituent of the reptilian gut microbiota, are potentially pathogenic and can become clinically important in times of stress. *Salmonella enterica houtenae* was previously isolated from reptiles and humans with pathological symptoms. However, this appears to be the first documented case in association with osteomyelitis in vertebrates.

INTRODUCTION

Infectious osteoarthropathy is frequently reported in reptile groups such as lizards, snakes and turtles (Silverman, 2006; Jacobson, 2007; Souza et al., 2014), however, detailed reports of osteomyelitis in crocodilians are lacking in the scientific literature. Osteomyelitis is defined as an inflammation of the bone marrow, cortex, and possibly periosteum. Although osteomyelitis is most commonly of bacterial origin, other causes can include fungi, parasites, viruses, foreign bodies, and corrosion of metal implants. Microorganisms typically gain access to bone through the bloodstream, localised infection, or trauma (Fossum & Hulse, 1992). Despite injuries often inflicted on crocodilians resulting from social disputes with other individuals in the wild and captivity, recovery usually occurs without infection in healthy animals. This is likely because their innate immune system reacts aggressively towards pathogens (Siroski et al., 2009). However, due to their shy, robust nature it can be difficult to distinguish sick individuals from healthy ones due to a lack of clinical signs, especially when submerged in water and as a result the animals often die before they can be treated (Leslie et al., 2011).

Salmonella bacteria are a common cause of osteomyelitis in reptiles (Souza et al., 2014). They are a common constituent of the reptilian gut microbiota and can be shed intermittently without displaying clinical signs. However, they are opportunistic facultative anaerobes that can become pathologically important to reptiles under stressful conditions (Jacobson, 2007). Reptiles living in captivity can experience stress more frequently than in wild populations, typically due to husbandry deficiencies.

Reports of *Salmonella enterica houtenae* date back to 1969 (Iveson et al., 1969) when it was isolated from a parasitic tick inhabiting the ear of a lizard. In 1978 it was isolated from the liver and air sac of a pet cockatiel (Phillips et al., 1978), and the authors suspected that it was transmitted from a wild reptile. They also refer to four samples submitted previously to the Veterinary Services Laboratories that were subsequently identified as

S. enterica houtenae. These samples were isolated from a snake in Texas and three snakes at the San Diego Zoo.

Salmonella bacteria have zoonotic potential. *S. enterica houtenae* has been implicated in a brain abscess in a 44 month old boy (Ma et al., 2003). The patient suffered a relapse but subsequently recovered. However, there was no verification of the patient or family coming into contact with any reptiles. In 2004, a 2.5 month old infant suffered meningitis (Wybo et al., 2004), *S. enterica houtenae* was reported as the pathogen responsible. This patient also suffered a relapse and later recovered. This family did, however, have two pet iguanas. Swabs were taken from the lizards but did not yield any pathogens. It is, however, possible that the lizards could have shed the bacterium at the time the swabs were collected. One lizard subsequently died.

Here, we report to the best of our knowledge the first known case of trunk vertebrae osteomyelitis in crocodilians. *S. enterica houtenae* was isolated from a direct swab of the vertebral lesions.

CASE REPORT

A privately owned female *Caiman crocodilus* ca. six years old was individually housed indoors in a 1,000 litre pond with 1.7 X 0.75 metres of land area available for basking. Temperatures were thermostatically controlled with ambient temperatures ranging from ca. 25˚C to ca. 30˚C with basking temperature ca. 35˚C. Water was filtered and maintained at 29˚C. UV lighting was provided. The caiman was fed on a diet of gut loaded invertebrates, fish, mice, chicken flesh and fresh water crustaceans. Multivitamin supplements were used occasionally.

After observing swelling and an exudative superficial excoriation on the right dorsum with damaged scales on the dorsal region of the right thigh, it was surmised that the caiman may have injured herself against the frame of her enclosure. The lesions were treated with pharmacy obtained iodine solution each day for several days. Judging that it was on its way to recovery, no further treatment was provided other than regular feeding and allowing the animal to thermoregulate freely.

After four months, the caiman stopped eating and was observed basking for prolonged periods of time in the terrestrial part of the enclosure. Drinking fresh water while on land was observed on two occasions. Over the following weeks, a mid-body swelling was observed and due to her mature age and change of season, in addition to her behaviour (lack of appetite/ increased basking), we suspected she was gravid. Two months later the swelling began to decrease, revealing a slight arching in the region of the twelfth and thirteenth trunk vertebrae with apparent kyphosis. No eggs were produced. The right dorsum was raised and a depression appeared on the left dorsum. The animal was admitted for veterinary investigation at the University College Dublin Veterinary Hospital.

Dorso-ventral and lateral radiographs of the whole body were taken. A survey computed tomographic (CT) helical scan of the whole body was performed using a four-slice CT unit with 1 mm slice thickness. The CT scan was reconstructed using bone and soft tissue algorithms (*Siemens Medical, Somatom Sensation 4, 2 Wittelsbacherplatz, 80333 Munich, Germany*). The animal was contained without sedation for all examinations. The radiographs and CT (Figs. 1a-1d) studies showed extensive muscle wastage and multifocal vertebral osteolytic changes with remodelling and subluxation between the twelfth and thirteenth trunk vertebrae. These findings were consistent with a multifocal disease with a pathological compression and fracture of the twelfth trunk vertebra. Based on the severity of the findings, the prognosis was considered poor and the caiman was euthanised.

The carcass weighed 5.2 kg, a decrease from ca. 8 kg in life, and appeared emaciated. Necropsy revealed 100 ml of free serosanguinous fluid within the coelomic cavity and a diffuse severe fibrinous coelomitis with firm adhesions between the intestines, liver and spleen. A 15 cm irregular mass with a firm exterior and bloody soft surface was found within and greatly expanding a major blood vessel ventral to the region of the twelfth and thirteenth trunk vertebrae. Examination of the vertebrae corroborated the severe osteolytic changes and remodelling as seen in the CT scan. The most severely affected vertebrae were the eleventh, twelfth and thirteenth (Figs. 1e & 1f). Histological examination of the twelfth trunk vertebra revealed a marked cellular infiltration of heterophils on a background of elongated cells with fusiform nuclei and indistinct cell borders (fibrosis). Macroscopic examination of the mass revealed an organised thrombus.

Bacteria were not detected on microscopic examination. This is common in bacterial osteomyelitis when endotoxaemia leads to thrombosis of metaphyseal vessels which results in ischemia and necrosis of bone (Silverman, 2006). However, a direct swab of the vertebral lesion was aseptically collected at necropsy and revealed *S. enterica houtenae*, *alpha haemolytic Streptococcus* and *Proteus* sp. The Salmonella strain was serotyped by slide agglutination and an analytical profile index was carried out to check the biochemical reactions revealing the antigenic formula 51:z4, z23. Further tests analysed the antimicrobial susceptibility of the strain and it was fully susceptible to all antimicrobials checked. For verification purposes, swabs were aseptically collected from the infected lesion, which was by this time, frozen and thawed during the post-mortem process. DNA was extracted using the ZR Fungal/ Bacterial DNA MiniPrep™ kit (Zymo Research, Irvine, California, USA). Universal bacterial primers were used to amplify the *16S rRNA* (Genewiz, USA). The isolates (five in total) were all identified using BLAST (Basic Local Alignment Search Tool) at NCBI (National Center for Biotechnology Information) as *Moellerella wisconsensis*, *Kurthia zopfii,* a *Vagococcus* sp., *Staphylococcus condimenti* and *Pseudomonas fragi*. There are no records of these bacteria causing osteomyelitis or disease in reptiles and it is likely that the reason they were detected after freezing was because the storage conditions may have altered the bacterial composition of the vertebrae.

DISCUSSION

To the best of the author's knowledge, this is the first report describing trunk vertebrae osteomyelitis in the order Crocodilia and we suggest *S. enterica houtenae* to be the causative organism. Due to their shy, robust nature, sick individuals often hide clinical signs and as a consequence, they can be difficult to identify (Leslie et al., 2011). In the case presented here, the severity of the imaging findings was surprising considering that the patient had no obvious ambulatory dysfunction. The radiographic studies grossly underestimated the skeletal changes due to the artefacts generated by the keratinised skin. However, the CT studies accurately demonstrated the severity of the problem and the very poor prognosis.

Salmonella enterica houtenae was previously isolated from reptiles and humans with pathological symptoms (Iveson et al., 1969; Ma et al., 2003; Phillips et al., 1978; Wybo et al., 2004). However, this appears to be the first documented case in association with osteomyelitis in vertebrates. Salmonella was the only pathogen isolated from the infected lesion that has been previously identified as the most common, definitive aetiological agent of osteomyelitis in reptiles (Jacobson, 2007; Souza et al., 2014), and we believe it is the likely pathogen responsible for the infection presented here. The bacteria likely came into contact with the caiman as a result of its captive environment. Crocodilians often thrash their food about in the water which could further aid in the contamination of the ambient environment. In this case, the injury previously sustained could have facilitated translocation of bacteria to the vertebrae, leading to a focus for infection, and ultimately causing terminal disease (Silverman, 2006; Jacobson, 2007). Therefore, this case should be considered as a possible trauma-related infection.

Salmonella is an important zoonotic organism widely associated with farming livestock and reptiles in the pet trade and zoos (Jacobson, 2007). It is a common cause of food poisoning and a source of more severe cases including a brain abscess, osteomyelitis and meningitis (Canessa et al., 2011; Ma et al., 2003; Wybo et al., 2004). Reptiles are intermittent shedders and as a result, Salmonella can be difficult to detect amongst individuals, therefore strict hygiene practises should be maintained when working or interacting with animals known to be natural carriers.

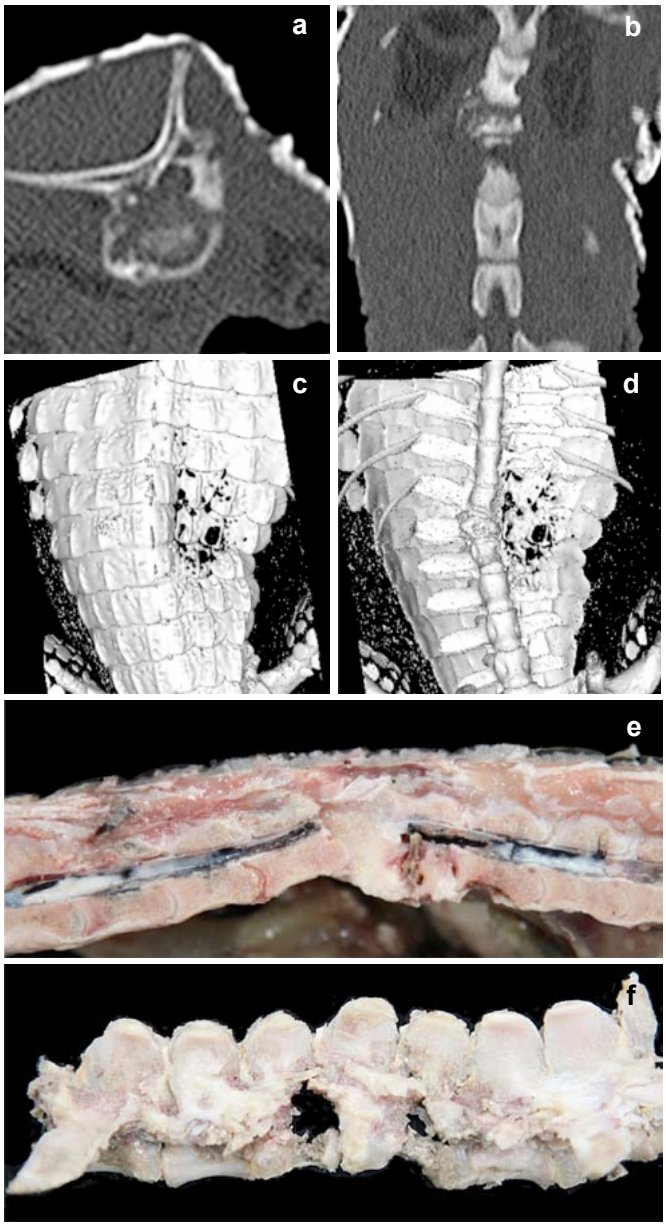

Figure 1a. Transverse CT image at the level of the twelfth trunk vertebra. The whole of the twelfth trunk vertebra and left lamina are remodeled with an amorphous shape and with patchy osteolytic changes. The spinous process is lytic and the left transverse process is missing. The depression of the epaxial muscle and the overlap of two areas of keratinized skin on the left side of the caiman are evident. **b.** Longitudinal 3D MPR reconstruction CT image of the caudal spine in the twelfth and thirteenth trunk vertebrae. There are severe osteolytic changes of the thirteenth trunk vertebra which shows an amorphous foreshortened and wedge shape. There is vertebral body lysis of the thirteenth trunk vertebra. The malalignment between the spine in the region of the twelfth and thirteenth trunk vertebrae is evident. **c.** Longitudinal 3D surface rendering reconstruction CT image of the caudal spine in the region of the twelfth and thirteenth trunk vertebrae. The depression of the left side of the paralumbar epaxial muscles and the curvature of the spine toward the left side are evident. **d.** Longitudinal 3D volume rendering reconstruction CT image of the caudal thoracic and lumbar spine. The vertebral body lysis of the last thoracic and first lumbar vertebral bodies are visible. There are lytic changes of the left transverse processes of the last three thoracic and first two lumbar vertebrae. The malalignment between the thoracic and lumbar spine is evident. **e.** Erosion of the vertebral bodies in the region of the twelfth and thirteenth trunk vertebrae. **f.** Obvious vertebral body erosion associated with infectious osteomyelitis.

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