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TITLE OF CASE ***Do not include "a case report"***

Osteomyelitis Caused By Beta Haemolytic Listeria Spp. In A Dog.

SUMMARY ***Up to 150 words summarising the case presentation and outcome (this will be freely available online)***

A 3.6 year old neutered female Labrador retriever was presented with one month's history of left hindlimb lameness and a 5 day history of anorexia. A severe osteolytic and osteoproductive polyostotic bone lesion affecting the proximal aspect of the tibia and the distal aspect of the femur was seen on radiographs. Histopathological evaluation confirmed cellulitis, myositis, neutrophilic osteomyelitis and bone necrosis and culture revealed a pure growth of beta haemolytic *Listeria spp.*. To the author's knowledge this is the first report of osteomyelitis caused by *Listeria spp* in a dog without any identified primary cause of infection.

BACKGROUND ***Why you think this case is important – why did you write it up?***

This case is the first report of osteomyelitis caused by *Listeria spp* in a dog. This case was a diagnostic challenge not only owing to the rarity of the disease but to also because the clinical and diagnostic features overlapped with polyostotic bone neoplastic disease.

CASE PRESENTATION ***Presenting features, clinical and environmental history***

A 3 year 7 months old female neutered 19 kg Labrador retriever dog was referred to the Queen's Veterinary School Hospital (QVSH), University of Cambridge for a one month history of left hindlimb lameness and a 5 day history of anorexia. On first presentation at the referring veterinarian 2 days after onset of 5/5 left pelvic limb lameness, a marked soft tissue swelling on the medial aspect of the stifle joint was noted and radiographs were taken. Conservative management with complete rest and non-steroidal anti-inflammatory medication (carprofren 4 mg/kg) was prescribed for presumptive diagnosis of medial collateral ligament injury.

On presentation at the QVSH, physical examination revealed a quiet mentation, a pyrexia of 39.5° C, a 5-8 % dehydration along with a non-weight bearing left pelvic limb lameness associated with muscle atrophy and severe soft tissue swelling and pain around the distal femur and proximal tibia. The popliteal lymph node was palpably enlarged. Neurological examination was normal.

INVESTIGATIONS ***If relevant***

Laboratory abnormalities included a mild lymphopenia (0.9 10^9/L, reference range 1-4.3), mildly increased globulins (52 g/L, reference range 24-47), and an elevated C-reactive protein (26.7 mg/L, reference range 0-8.2).

Cranio-caudal and lateral radiographs of the left stifle (Celtic SMR Nova HF, 50 KVp, 5 mAs) revealed a severe soft tissue swelling surrounding the entire stifle joint, a markedly osteolytic lesion involving the proximal aspect of the tibia and tibial tuberosity, with thinning of the cortices, a small radiolucent line extending distally from the tibial plateau suspicious of a pathological fracture, and some sub-lesional sclerosis of the medullary cavity. The femur showed a predominantly lytic lesion in the distal diaphysis/proximal metaphyseal region surrounded by a large amount of smoothly marginated periosteal reaction and some evidence of medial cortical destruction. In the most proximal aspect of the femoral shaft, a small amount of periosteal reaction was also noted cranially. An enlarged popliteal lymph node was also noted. Compared to

the radiographs obtained by the referring veterinarian three weeks prior to presentation, a dramatic increase in the osteolytic changes was seen. Figures 1 and 2 show the radiographic results.

Radiographs of the right stifle, pelvis, thorax and lumbar spine were unremarkable.

Fine needle aspirates (FNA) from the femoral and tibial lesions revealed neutrophilic inflammation (>90% neutrophils), with lower numbers of activated macrophages with leukophagia and occasional small lymphocytes, plasma cells, mast cells and eosinophils. The presence of intracellular rod shaped bacteria was also noted. Mesenchymal cells (including presumed osteoblasts and fibroblasts) demonstrating moderate criteria of atypia (moderate anisocytosis and anisokaryosis, mildly increased nuclear:cytoplasmic ratio, prominent nucleoli and occasional binucleation) were also noted. Figure 3 illustrates these findings.

An abdominal ultrasound (Phillips HDI 5000 Sono CT System, Netherlands, microconvex probe 5-8 MHz) revealed only one abnormality: an enlarged hypoechoic left medial iliac lymph node (1.4 cm diameter, contralateral lymph node 0.47 cm diameter). Cytology of the left medial iliac lymph node revealed neutrophilic lymphadenitis with 15 % neutrophils, an increased number of plasma cells (> 30 %) and 40 % small and 15 % intermediate to large lymphocytes.

Ultrasound of the left stifle revealed a diffuse soft tissue swelling surrounding the stifle, without evidence of a discrete periarticular soft tissue mass, and a marked irregular cortical surface of the proximal tibia and distal femur.

Histology performed via Tru-cut and Jamshidi biopsies of the bone lesions and surrounding soft tissues was consistent with fibroplasia, with chronic lymphocytic plasmacytic neutrophilic and histiocytic multifocal periarthritis and multifocal moderate bone necrosis.

Urine analysis showed a mild elevation in UPC (0.7; reference range 0-0.4), a urine specific gravity of 1.025, a pH of 8.5 and an inactive sediment. Serum protein electrophoresis was consistent with an acute phase response.

Echocardiography was unremarkable; in particular there were no evidence of vegetations or thickening of the cardiac valves.

Cultures from the bone and medial iliac lymph nodes revealed a sparse pure growth of beta haemolytic *Listeria spp.* (likely *Listeria monocytogenes*). This was sensitive to ampicillin, co-amoxyclav, cephalexin, co-trimoxazole and oxytetracycline, but resistant to enrofloxacin, metronidazole and clindamycin. A fungal culture was performed and showed no growth after two weeks.

DIFFERENTIAL DIAGNOSIS ***If relevant***

Aggressive bone lesions are characterized radiographically by one or a multiple of the three following criteria of aggressiveness: an indistinct transition zone between the lesion and the adjacent normal bone, the presence of cortical destruction and an active periosteal reaction. Whether the lesion is predominantly osteolytic or sclerotic has no bearing on the aggressive nature of the lesion (Thrall, 2013). Polyostotic aggressive lesions are likely to be malignant and have a narrow list of differentials; essentially neoplasia (primary or secondary), osteomyelitis or immune mediated diseases such as erosive polyarthritis.

At this point, our two main differentials were an aggressive neoplasm (primary or secondary in origin) or a primary osteomyelitis as our samples suggested. Neoplastic disease could not be ruled out as the only samples of tissue obtained were from Tru-cut and Jamshidi biopsies, which are inherently small and thus may be representative of the whole lesion.

TREATMENT ***If relevant***

Owing to the extent of the lesions, surgical treatment and concurrent medical treatment of the presumptive osteomyelitis or limb amputation were considered. The owners elected for limb amputation.

OUTCOME AND FOLLOW-UP

On macroscopic pathological examination, the soft tissues and musculature overlying the proximal tibia showed a focal area containing dark yellow/pale brown mildly viscid material (purulent exudate), and areas of irregular thickening overlying the cortical bone, consistent with periosteal new bone formation as shown in Figure 4. The joints and cartilage did not demonstrate macroscopic lesions

Histologically, the muscles overlying the distal femur and proximal tibia contained a perivascular to diffuse infiltrate composed by large numbers of neutrophils and fewer macrophages and some areas of haemorrhage. In the affected bone, multifocally surrounding irregular scalloped bone trabeculae within the medullary cavity there were moderate to large numbers of neutrophils and, in a multifocal to coalescing distribution and often surrounding fragments of necrotic bone, proliferation of fibroblasts was present. There was also evidence of a pathological fracture on the articular surface of the tibial plateau. Areas of periosteal new bone formation were also observed. The overall histological diagnosis was cellulitis and myositis along with neutrophilic osteomyelitis associated with bone necrosis and periosteal new bone formation. Figure 5 shows the histology results.

The dog was prescribed a 10 days course of oral cephalexin at 20 mg/kg BID (Rilexine, Virbac, UK) and oral non-steroidal anti-inflammatory medication (carprofen: Rimadyl, Zoetis) for 7 days. Prior to discharge, the left medial iliac lymph node was noted to have decreased to half of its original size on ultrasound. One month after surgery, the patient is doing well and has not shown any clinical signs.

DISCUSSION ***Include a very brief review of similar published cases***

Differentiating malignant bone neoplasia form osteomyelitis can be difficult. Neoplastic lesions often have a higher degree and extent of osteolysis, a more irregular periosteal reaction with a spiculated radiating pattern, and more often display a triangular area of new subperiosteal bone originating from periosteal elevation away from the bone, also known as a Codman triangle (Dennis et al., 2010). Polyostotic osteomyelitis is more likely to be fungal than bacterial in origin, especially in areas where predisposing fungi are endemic (Kevin Kealy et al., 2011), is usually associated with more soft tissue swelling than neoplasia. Sequestrum formation may occur with osteomyelitis but not neoplasia (Dennis et al., 2010). More rapid bone changes tend to occur with neoplasia than osteomyelitis (Robert, 2000).

Osteomyelitis can originate from haematogenous spread, extension from a surrounding infective area or can be post traumatic. The post-traumatic form is the most common form seen in small animal practice, from direct inoculation of bacteria (Rabillard et al., 2011). Staphylococcus is isolated in 70 % of the cases (Stead, 1984) (Caywood et al., 1978). Other bacterial and fungal agents have also been reported (Caywood et al., 1978).

An interesting finding in this case is the presence of a pathological fracture, both seen on the radiographs and on the histopathological samples. Neoplasia usually accounts for 93 % of cases of pathological fractures (Boulay et al., 1987), and fractures originating from haematogenous osteomyelitis have only been reported in one case report (Emmerson and Pead, 1999) and presumed in 4/67 dogs in another study, of which only one was polyostotic (Caywood et al., 1978)

Haematogenous osteomyelitis generally occurs in young (Dunn et al., 2008) or immunosuppressed animals and accounts for 2.8 to 10 per cent of all cases. There are very few reports in adult dogs (Rabillard et al., 2011) (Emmerson and Pead, 1999). One report (Rabillard et al., 2011) describes three dogs with haematogenous osteomyelitis of bacterial origin in which *Streptococcus canis*, *Bacteriodes spp*, *Fusobacterium spp*, *Priprionibacterium acnes* and *Gemella morbillorum* were isolated. Similar to humans (according to the NHS guidelines), treatment for

haematogenous osteomyelitis in dogs is usually a combination of long course of antibiotics, surgical debridement, drainage and copious lavage, and surgical repair of pathological fractures (Tobias and Johnston, n.d.). In this study of three dogs, two dogs were successfully treated with antibiotics and the most severely affected dog with polyostotic bone lesions was euthanized after extensive therapy. A second report (Emmerson and Pead, 1999) describes a case associated with a pathological fracture which healed without complication after internal fixation and antibiotic therapy. Neither of these two dogs showed severe systemic signs, contrary to the patient in this case report.

Listeria monocytogenes is a facultative anaerobic, gram positive, rod shaped bacteria. Clinical disease attributed to Listeria is rarely reported in dogs. The bacteria usually affect sheep and cattle, causing influenza like signs, meningo-encephalitis, septicaemia and abortion or stillbirth. One case of cutaneous listeriosis (Loncarevic et al., 1999) is described in a dog showing multiple pustules. Two cases of tonsillitis (Läikkö et al., 2004), one case of septicaemia (Pritchard et al., 2016), one case of a urinary tract infection in an immunocompromised dog (Palerme et al., 2016), one case of encephalitis (Chapman, 1947) and one generalised infection (Schroeder and van Rensburg, 1993) by *Listeria monocytogenes* have been described in the literature.

The origin of the infection may be contaminated food. In one study, *Listeria monocytogenes* was isolated from 16 % of tested raw food samples, and 14 % of other samples were confirmed for other Listeria species (Nemser et al., 2014). Direct contact with the carcass or faeces of wildlife may also be an infection source as the bacteria were reportedly isolated from 15 % of the faeces of wild animals (Weis and Seeliger, 1975). Finally, Listeria may infect an immunocompromised patient as it has been isolated as part of the normal intestinal flora from the faeces of apparently healthy dogs (15.6%) and cats (7.4%) (Loncarevic et al., 1999).

Osteomyelitis caused by *L. monocytogenes* has never been reported in dogs. In people, it has been reported occasionally, in two cases in one study (Chirgwin and Gleich, 1989) and in 43 consecutive cases in another study (Charlier et al., 2012). However it was always associated with either the presence of a prosthesis, an underlying systemic disease commonly associated with

decreased immunologic responsiveness such as diabetes or rheumatoid arthritis or treatment with immunosuppressive drugs such as steroids.

In our case, the source of the infection remains unknown. The patient was fed a commercially available wet and dry diet, did not show any signs of immunocompromise, had never had any form of trauma or surgery involving the placement of an implant or prosthesis and was not on any immunosuppressive medications. Hence haematogenous spread is the most likely route of infection. The site of infection is also a feature which is suggestive of haematogenous osteomyelitis in this patient. The zoonotic potential for this patient is unknown.

In conclusion, although an uncommon presentation, osteomyelitis can be caused by *Listeria monocytogenes* in adult, immunocompetent dogs, can present in a very similar fashion to polyostotic neoplastic processes and therefore should be considered a differential diagnosis.

LEARNING POINTS/TAKE HOME MESSAGES ***3 to 5 bullet points – this is a required field***

- Osteomyelitis caused by *Listeria monocytogenes* is reported for the first time
- This disease can occur in adult immunocompetent dogs without an identifiable cause of infection
- The presentation of this disease can be very similar in fashion to polyostotic neoplastic processes

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FIGURE/VIDEO CAPTIONS ***figures should NOT be embedded in this document***

Figure 1: Latero-medial and cranio-caudal radiographs of the left stifle. Far left: At initial presentation at the referring veterinarian. Middle and right: First presentation at our hospital. Marked soft tissue swelling, osteolysis and osteoproduction in the femur and tibia. The lesions are faintly visible on the initial radiograph.

Figure 2: Close up view of the lateral stifle radiograph. Black arrow indicates a radiolucent line, representing a possible pathological fracture.

Figure 3: Figure 3: FNA cytology from the femoral lesion: neutrophilic inflammation, intracellular rod shaped bacteria and a large reactive mesenchymal cell. The background shows a granular appearance due to the proteinaceous nature of the sample. Thin arrow: neutrophil with intracellular rods. Arrowhead: large mesenchymal cell. A close up of the neutrophil with

intracellular rods is also provided. Wright's Giemsa, 50 x objective.

Figure 4: Longitudinal section of the distal femur: thickened irregular areas of periosteal new bone formation adjacent to the cortical bone. Black star shows the periosteal new bone elevation. Green star indicates the exudate.

Figure 5. Femur. Multifocally irregular cortical bone (A and B), lined by plump, reactive osteoblasts (A, black arrow) and occasionally osteoclasts (B and C, arrow head). Large numbers of neutrophils are present adjacent to cortical bone (B) and within the medullary cavity (B, inset photograph). Within the medullary cavitary there are fragments of necrotic bone (D, white arrow), multifocally surrounded by fibrous tissue (D, inset photograph).

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