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

An unusual presentation of Actinomycosis in a dairy cow

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

A first lactation dairy cow (in late gestation) presented with a hard round swelling of the right olecranon and slight lameness. A lateromedial radiograph of the right olecranon had a mottled appearance with indistinct borders.

Bone biopsy was performed post-calving. Histology showed fibrosis and reactive changes, however this sample was considered too superficial and not representative of the whole lesion.

Based on the differential diagnoses treatment was not possible and the cow was culled at the end of her lactation. At slaughter the forelimb was retained for further analysis. Grossly, the olecranon was severely enlarged, irregular with multiple yellow granulomas in the cortex and medulla of the bone. Histology showed multifocal pyogranulomas containing bacteria, Splendore-Hoeppli material and surrounded by remodelled bone and fibrosis.

Despite displaying biochemical properties characteristic of *Actinomyces spp* this could not be confirmed by partial 16s rRNA sequencing and the organism could not be definitively speciated.

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

Actinomyces sp, are responsible for a wide range of animal and human infections (Table 1). In cattle, actinomycosis- mainly caused by *Actinomyces bovis*- most commonly affects mandibular and maxillary bones due to its role as a commensal of the respiratory tract, and is frequently isolated in the region of the oesophageal groove or anterior wall of the reticulum (Radostits, 2007). This case report outlines an unusual presentation of this disease, and highlights that it should remain on the differential diagnosis list for bony swellings in the appendicular skeleton.

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

The heifer presented at 34 months of age with a hard round swelling of the olecranon process of the right ulna measuring 8cm, and moderate atrophy of the triceps brachii muscle. There was no palpable joint effusion. She showed slight lameness with no behavioural changes, judged as 3/5 by the Manson and Leaver system of locomotion scoring (Channon 2009). Mobility scoring was not routinely carried out, so previous locomotion scores were not available. She preferred not to weight bear when stationary, instead resting her toe on the ground. The farmer had not previously noticed the lameness, but the degree of muscle atrophy and the size of the swelling would suggest this had been developing for at least 3-4 weeks. She was part of an 80 cow Holstein-Friesian dairy herd housed in cubicle housing and milked using robots. She was in the dry period after completing her first lactation, one month prior to calving.

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INVESTIGATIONS ***If relevant***

Lateromedial radiographs were taken of her right elbow (80kV, 2.0mAs). To avoid use of potentially abortifacient sedative, the heifer was restrained standing in a crush. It was therefore not possible to obtain other radiographic views. The olecranon showed diffuse multifocal regions of lysis with destruction of the cortex and marked periosteal reaction (Plate 1; Fig. A).

Diagnosis was pursued using bone biopsy in order to inform prognosis. The area was clipped and surgically scrubbed using chlorhexidine (Hibiscrub, Mölnlycke Health Care) and then surgical

spirit. Thirty millilitres of local anaesthetic (Adrenacaine, Norbrook, procaine and adrenaline) was infiltrated into the region and a core biopsy attempted using a trephine. The heifer quickly resented the pressure applied to the lesion, and therefore only a small sample was obtained. The incision site was closed with cruciate sutures using monofilament nylon (Ethilon, Ethicon, USA). Unfortunately the sample included only connective tissue and no bone, and was therefore insufficient for histological diagnosis of the bone lesion.

A fortnight after calving bone biopsy was re-attempted. The cow was sedated using 0.1mg/kg 2% xylazine intravenously (Sedaxylan, Dechra veterinary products, Shrewsbury), followed by 3mg/kg ketamine (Ketamidor, Chanelle UK) intravenously. .The cow was cast using Reuff's method, the elbow region was surgically prepared, and 30ml procaine (Adrenacaine, Norbrook) was infiltrated. A trephine biopsy was attempted; but the trephine was unable to grip the bone sufficiently. Despite heavy sedation (including two ketamine "top-ups"), this stimulated the cow to rise to sternal recumbency making sampling more difficult. Instead a bone chisel and mallet were used to remove a bone chip for histological analysis, which was preserved in 10% buffered formalin. Due to the difficulty of sampling, no bone was available for bacterial culture. Histology showed again prominent fibrosis with few mixed inflammatory infiltrates with no evidence of neoplasia.

DIFFERENTIAL DIAGNOSIS ***If relevant***

Lameness with palpable bone abnormality most likely indicates a fracture, bone neoplasia or osteomyelitis. There was no evidence of fracture on the radiograph. Osteomyelitis in cattle may be caused by local trauma, direct spread of infection from neighbouring tissues, or by haematogenous spread of bacteria. The latter is most common, with metaphyses most frequently affected as they contain few mononuclear phagocytes, and blood flow is slow in metaphyseal sinusoids (Verschooten et al 2000). In this case there was no history or evidence on clinical examination of trauma or infection of the adjacent joint or soft tissues. Haematogenous spread seemed unlikely as this is most common in young calves, and there was no identifiable source of infection (Desrochers 2014).

Neoplasia was therefore considered more likely, however this was ruled out by histopathology, leaving osteomyelitis as the primary differential.

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TREATMENT ***If relevant***

The heifer received two treatments of 0.5mg/kg meloxicam (Metacam, Boehringer Ingelheim) on initial presentation, and repeat doses following bone biopsy attempts.

OUTCOME AND FOLLOW-UP

Despite the poor prognosis, it was not felt that culling immediately was necessary for welfare reasons, so the farmer continued to monitor the cow for signs of progression. He reported the swelling appeared to be growing, so repeat radiographs were taken. These confirmed the lesion had grown larger, and the radiographic features had changed. The borders of the olecranon process were no longer distinct. It was advised that she should not be served again.

The cow was culled at the end of her second lactation and the forelimb collected from the abattoir for further investigation. No other abnormalities in the carcase were noted by the meat inspector or veterinary inspector in the abattoir. Post-mortem lesions showed a severe diffuse osteomyelitis of the right olecranon with multiple pyogranulomas (Plate 1; Fig. B); severe diffuse fibrosis with multifocal pyogranulomas in the ligaments and tendons associated with the right elbow and fibrosis of the right triceps brachii muscle. Histopathology was predominated by bone remodelling, which was also observed grossly (Plate 1; Fig. C and D), fibrosis and multiple pyogranulomas centred on rosette-like bacterial colonies surrounded by Splendore-Hoeppli material (Plate 2; Fig A and B) suggesting *Actinomyces* sp. as the cause of the osteomyelitis lesion. Bacterial culture was performed using material from the lesion plated on sheep blood agar plates (Oxoid, UK) with CO_2 added, and on Fastidious Anaerobe agar (BD, UK) incubated anaerobically at 37°C. Presumptive phenotypic identification was performed by Gram staining, Modified ZN stain, evaluation of the colony morphology, and catalase reaction. Biochemical testing was performed with API biochemical kits API Rapid ID32Strep and API Rapid 32A, according to the manufacturer's instructions (bioMerieux, FR). The cultured organism consisted of Gram-positive, cocco-bacillary to short diphtheroid-shaped rods, which were non-acid-fast and non-spore-forming. After 48 h anaerobic incubation on Fastidious Anaerobe Agar with 5 % horse blood, colonies were < 1 mm diameter, convex, smooth, entire-edged, white and β-haemolytic. The organism was catalase-positive and grew poorly in air, and in air plus 10 % CO_2, but grew well in anaerobic conditions. On the basis of these results the organism was presumptively identified as *Actinomyces* sp. As determined by traditional methods, the organism produced acid from D-ribose, mannitol, sorbitol and trehalose and D-xylose. The organism hydrolysed aesculin but not gelatin or starch. It did not produce lecithinase, lipase or urease, was indole- negative and did not reduce nitrate. The strain could not be identified using the API biochemical kits.

Partial 16s rRNA gene sequencing was performed as described by Randall et al. (2015), revealing 93% similarity with *Actinomyces howellii* a species isolated from bovine dental plaque (Dent and Williams, 1984) The 16s sequence was submitted to the NCBI database (Genbank accession number KY271343).

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

Although neoplasia had been ruled out in the live animal and osteomyelitis seemed the most likely diagnosis, *Actinomyces* sp. had not been specifically considered as a causative agent. Although there are numerous published cases reporting *Actinomyces bovis* in the mandible and head regions (Jubb et al. 2016, McGavin et al. 2012, Tessele 2014), and one report of tracheal actinomycosis in a cow (Bertone 1984), to our knowledge this is the first report of actinomycosis in the appendicular skeleton.

The most likely aetiology of this lesion was considered to be local trauma such as a previous contaminated puncture wound. No other lesion related to a possible contamination via blood was found either on clinical examination or during abattoir inspection. Moreover, the haematogenous route of dissemination usually results in multifocal lesions. Farm records did not indicate previous disease or antibiotic treatment in this animal since weaning. Disease pre-weaning cannot be ruled out as records are kept according to collar number (for automatic milk feeding machines)

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which is unrelated to post-weaning identification.

Treatment options for this case were very limited. Long-term anti-inflammatory treatment is not economic in lactating dairy cows. The products available with nil milk withhold require daily injections, and would be prohibitively expensive. Treatment for chronic osteomyelitis could have been attempted by regional limb perfusion or regional osseous perfusion with antibiotics. These techniques certainly have the ability to deliver high concentrations of antimicrobials to a targeted site (Clegg 2011), however the benefits of such treatment in actinomycosis cases is unknown, particularly without the possibility of surgical debridement of the lesion. In any case, a clinical judgement was made that the lesion was too extensive to respond to intramuscular antibiotic treatment, which would have been the only economically viable option.

This case illustrates the practical difficulties of obtaining bone biopsies from cattle in the field. On reflection, periosteal instillation of local anaesthetic may be beneficial for improving cow compliance. It may be that general anaesthetic is the best solution.

Microbiological identification of *Actinomyces* to the species level is difficult in the clinical laboratory. Even with biochemical tests such as the RapID ANA II test, whole-cell fatty acid analysis, or gas-liquid chromatography, assignment to a species is difficult (Clarridge and Zhang, 2002). Thus, based only on Gram staining, the catalase reaction, and better growth under anaerobic conditions than aerobic conditions, strains may be assigned to the genus *Actinomyces*.

The low similarity found with *A. howelli* 16s ribosomal DNA and the biochemical profile not matching any of the described catalase positive *Actinomyces* species listed in Table 1 did not allow this isolate to be assigned to a particular species inside the *Actinomyces* genus. It may be of interest to note that identification of *A. howellii* is prone to difficulties due to the fact that only some biochemical reactions to a few strains were reported (Dent and Williams, 1984). The use of 16s rDNA sequence analysis has proven to have some limitations in the identification of *Actinomyces* spp., in a study investigating 16s rDNA of putative *Actinomyces* spp. and only 70% of the strains were clearly assigned to recognized *Actinomyces* species (Hall et al., 2001).

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

- Consider periosteal instillation of local anaesthetic or even general anaesthesia for taking bone biopsies from adult cows
- Consider *Actinomyces spp* as a cause of radiographic lesions with mottled appearance in the appendicular skeleton in cattle
- Consider actinomycosis as a differential diagnosis for bony swellings in the appendicular skeleton
- Consider *Actinomyces howelli* as a cause of osteomyelitis in cattle

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

Plate 1. Fig. A: Lateromedial radiograph of the olecranon showing diffuse multifocal regions of lysis with destruction of the cortex and marked periosteal reaction; Fig B: cross section of the olecranon showing severe diffuse chronic osteomyelitis with multifocal yellow pyogranulomas and scattered haemorrhages; Fig C and D: lateral and medial aspect of the olecranon after removal of soft tissues showing severe bone remodelling with haphazardly anastomosing osseous trabeculae.

Plate 2. Fig A: Soft tissues and joint capsule of elbow showing diffuse fibrosis with multifocal rosette-like aggregates of bacterial elements and pyogranulomatous inflammation; Fig B: Closer view of a bacterial colony showing abundant amorphous hypereosinophilic material and cocco-bacillary bacteria surrounded by Splendore-Hoeppli material and characteristic pyogranulomatous inflammatory infiltrates .

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