Diffuse osteomyelitis of the fourth metacarpal bone in a horse caused by *Clostridium perfringens*

C.E. Smith [†], M.D. Cullen [†], I.M Forman [‡], A.M. Talbot[†], J.D. Stack [†]

[†]Philip Leverhulme Equine Hospital, School of Veterinary Science, Leahurst, Neston, Cheshire

[‡]Beechwood Vets, Kidsgrove, Staffordshire

Summary

Osteomyelitis in adult horses, often associated with trauma or iatrogenic infection following surgery, usually presents as a focal area of infected bone. Diffuse osteomyelitis, affecting both the cortex and medulla, along the full length of a bone is encountered less frequently and treatment of such infections is not well reported in horses. The two-year-old Warmblood mare in this case was diagnosed with diffuse osteomyelitis affecting the 4th metacarpal bone with concurrent unicortical fracture of the third metacarpal bone following traumatic injury. Computed tomography (CT) aided diagnosis in this case, providing superior information compared to radiography and ultrasound. This case highlights the value of CT in the diagnosis of diffuse osteomyelitis. This is the first reported case of diffuse osteomyelitis caused by *Clostridium perfringens* in horses. Successful treatment in this case consisted of surgical debridement of the associated abscess, followed by systemic and locoregional antimicrobial therapies.

Keywords

osteomyelitis; splint bone; clostridial; computed tomography; metacarpal

Introduction

Osteomyelitis is a well-recognised but infrequently reported condition in mature horses (Goodrich, 2006, Sayegh et al., 2001). The vast majority of cases are either traumatically induced, with direct bacterial inoculation of bone, or associated with iatrogenic infection following surgery (with or without the presence of metallic implants) (Goodrich, 2006). Osteomyelitis caused by trauma often results in focal osteomyelitis with sequestration of necrotic, avascular bone (Gibbs, 1994). Focal osteomyelitis is frequently treated with removal of infected implants, surgical debridement of necrotic bone and removal of sequestra, along with regional and systemic antimicrobial therapy (Goodrich and Nixon, 2004).

Diffuse osteomyelitis, is defined as widespread infection of cortical and medullary bone affecting a large proportion of its length, resulting in a loss of stability (Lazzarini et al., 2004). In humans, this distribution of infection in an otherwise healthy individual is classified as stage 4A osteomyelitis (Cierny 3rd et al., 2003, Lazzarini et al., 2004) (Table 1). Despite their superficial location, and the frequency of traumatic injuries, there are surprisingly few reports of diffuse osteomyelitis affecting the long bones of the appendicular equine skeleton and, therefore, evidence-based treatment recommendations are lacking (Sayegh et al., 2001, Goodrich, 2006). Common bacterial species cultured from osteomyelitis in adult horses include *Enterobacteriacea*, *Streptococcal* and *Staphylococcal* species (Moore et al., 1992; Goodrich, 2006; Gieling et al., 2019).

This report describes the diagnosis by computed tomography (CT) and successful treatment of diffuse osteomyelitis of the fourth metacarpal bone (MCIV) caused by *Clostridium perfringens*. This occurred concurrent to a unicortical fracture of the adjacent third metacarpal bone (MCIII), following a focal traumatic injury.

Case history

A 2-year-old Warmblood mare presented for further investigation of chronic left forelimb cellulitis and lameness. A small focal wound on the dorsolateral aspect of the left mid-metacarpal region had been detected two weeks previously by the owner. The horse was thought to have rolled over onto the slightly raised metal rim of a drain cover in the field. Prior to referral, veterinary management consisted of systemic antimicrobial (ceftiofur 2.2mg/kg IV q12 hours, enrofloxacin 7mg/kg IV q24 hours) and anti-inflammatory (phenylbutazone 2.2 mg/*kg per os* q12 hours) medications administered over 7 days, and surgical exploration of the region with superficial curettage of the dorsolateral aspect of MCIII. Bacterial culture was not attempted. There were no significant bone changes on radiographs obtained by the referring veterinarian (Figures 1 and 2, Day 7). Despite this treatment regime, lameness and swelling persisted, with no improvement reported at the time of referral.

Clinical findings

On presentation the mare was bright and alert, but was underweight with a body condition score of 4/9 (Henneke et al., 1983). There was moderate, diffuse swelling of the left distal forelimb, though no lameness was observed at walk. A 3cm long vertical skin wound, the site of the previous surgical exploration, was present on the lateral aspect of the left fore proximal metacarpus. Palpation of the lateral aspect of metacarpal region elicited moderate discomfort. Visible and palpable effusion of the middle carpal joint was noted.

Further investigation

Ultrasound examination (GE Vivid 7 Ultrasound)[1] with a high frequency linear transducer (MHz -15)[1] of the carpus and metacarpal region revealed multiple shallow pockets of heterogeneous material in the subcutaneous tissues on the dorsolateral aspect of the mid-metacarpal region. The periosteum of MCIV was diffusely irregular. Proliferative periosteum extended proximal and distal from an area that was devoid of periosteal reaction at the level of the wound. The bone surface of MCIII was regular and smooth, as was the junction between MCIII and MCIV. No sequestrum or foreign bodies were identified. There was mild anechoic effusion of all 3 carpal joints. Centesis of the middle carpal joint yielded normal synovial fluid (nucleated cell count 0.7 $x10^9$/l; total protein 3.4g/l). A deep swab was taken from the wound and submitted for bacterial culture.

Standard radiographic projections of the metacarpal region were obtained using a digital wireless system (Canon CXDI-801C)[2]. Radiographs confirmed the presence of palisading new periosteal bone along the abaxial margin of MCIV and associated moderate soft tissue swelling (Figure 1, Day 18). Based on these findings, computed tomography (CT) under general anaesthesia was recommended to identify a cause for the refractory cellulitis, such as a radiographically occult fracture of MCIII or MCIV, sequestrum, foreign body or osteomyelitis.

The horse was pre-medicated with acepromazine (0.03 mg/kg IM), romifidine (0.04 mg/kg IV) and morphine (0.2 mg/kg IV). General anaesthesia was induced with ketamine (2.2mg/kg IV) and diazepam (0.05mg/kg IV). Two further doses of ketamine (500mg IV) were administered to maintain general anaesthesia for the duration of the CT scan.

Computer tomography was performed using a helical 16 slice Aquilion large bore sliding gantry (Cannon medical systems, Zoetemeer, Netherlands)[3] with the horse in right lateral recumbency with the left forelimb extended through the isocentre of the bore. Contiguous transverse scans were obtained with 2mm diameter and 1mm overlap using a 175mm field of view and exposure settings of 120kVp and 300mAs (Figures 3, 4, 5). Computed tomography identified a semicircular hypoattenuating bone discontinuity, consistent with unicortical, Y-shaped fracture of the palmarolateral aspect of the third metacarpal bone adjacent to MCIV at the level of the nutrient foramen (Figure 4, 5). This fracture configuration resulted in a minimally displaced fragment of cortical bone (measuring 27mm x 5mm x 9mm), immediately deep to the syndesmosis with MCIV. The trabecular bone throughout the medulla of MCIV was hypoattenuating, and the cortex of MCIV was multifocally hypoattenuating. There was abundant periosteal new bone formation abaxially along MCIV apart from a short (8mm) region, level with the scar of the original wound (Figure 4, black elipse).

Diagnosis

Y-shaped cortical fracture of the lateral cortex of MCIII and diffuse, stage 4A (Cierny 3rd *et al*, 2003) osteomyelitis of MCIV.

Treatment

Following CT, the horse was transferred to the surgical suite, where general anesthesia was maintained with inhaled isoflurane. The wound edges and subcutaneous pockets were sharply debrided and lavaged. A sample of periosteum from MCIV was obtained using 2mm Ferris Smith Rongeurs (Storz)[4] and submitted for bacterial culture in enrichment broth (Signal Blood culture system, Oxoid LTD)[5] and direct Gram smear. The wound was partially closed proximally using 3.5M polypropylene (0 USP) simple interrupted sutures, and an intravenous regional limb perfusion (IVRP) was carried out with amikacin (500mg). The mare received phenylbutazone (4.4 mg/kg IV) at anaesthetic induction which was continued until hospital discharge (2.2 mg/kg IV q12 hours). Treatment with oxytetracycline (7.5mg IV q12 hours) was instituted immediately following periosteal sampling. A full limb cast was placed and following uneventful rope-assisted recovery replaced with Robert jones bandage. The horse was maintained in a Robert Jones bandage and IVRP with amikacin was repeated on alternate days (n=2). No bacterial growth was identified from the wound swab or periosteal tissue samples and no bacteria were seen on direct Gram smear of the periosteal tissue. The mare was discharged four days postoperatively on oral doxycycline (10mg/kg *per os* q12 hours) and phenylbutazone (2.2 mg/kg PO q12 hours). At discharge no lameness was evident at walk and the wound was well apposed with minimal discharge.

Clinical and diagnostic findings (2nd presentation)

Four days following hospital discharge, the mare re-presented due to increasing discomfort and development of acute swelling from the left carpus distally. The horse was 4/5 lame (AAEP lameness scale 1991) and scored 10/12 on the horse grimace scale (Dalla Costa et al., 2014). Palpation of the surgical wound yielded purulent discharge. A sample was taken for bacterial culture, which yielded a heavy growth of *Clostridium perfringens*. Haematology revealed no significant abnormalities; however, serum amyloid A (SAA) was marginally increased (36.6mg/l). Repeat ultrasound revealed no additional significant findings. Radiography showed a markedly heterogeneous appearance of MCIV with marked periosteal new bone formation and multifocal lysis of the cortex, consistent with osteomyelitis affecting most of the bone's length (Gibbs, 1994; Baxter 1996) (Figure 1, Day 28).

Treatment and outcome

Due to marked initial discomfort, both phenylbutazone (4.4 mg/kg) and paracetamol (20mg/kg) were administered. In addition, a one-off intramuscular dose of morphine (120mg) was administered. All sutures were removed to facilitate drainage and the wound was lavaged. Gentamycin-impregnated polymethylmethacrylate (PMMA) beads were inserted into the wound pocket and intravenous regional perfusion (IVRP) was carried out using amikacin (500mg) before bandaging the limb. Enrofloxacin was administered systemically (7.5mg/kg *per os* q24 hours) and IVRP repeated daily (n=4). All IVRPs were performed either via the cephalic or medial palmar vein following median and ulnar nerve blocking (15 ml of mepivacaine per nerve). Once culture results revealed the presence of *Clostridum perfringens*, the horse was given metronidazole (15mg/kg *per os* q8 hours), metronidazole-soaked gauze swabs were placed within the wound pocket (replacing the gentamycin PMMA beads) and IVRP was carried out daily with ceftiofur (500mg) (n=3). Local treatment was discontinued after 7 treatments as the horse was sound at walk and the wound was dry and starting to heal. The lower limb was maintained in a bandage and was changed every two days.

The mare was discharged after a further eight days and managed on enrofloxacin, metronidazole, and tapering courses of phenylbutazone and paracetamol. Enrofloxacin treatment was discontinued four weeks post hospital discharge and metronidazole after a further four weeks. The horse was confined to a stable for 10 months with grazing in hand permitted during the third month and an in hand walking programme instituted in month 4 consisting of 5 minutes walking daily with weekly incremental increases of 5 minutes. The wound had healed fully by four weeks after hospital discharge and the there was no evidence of lameness at walk by 8 weeks following hospital discharge. Ten months following diagnosis some mild swelling of the proximolateral aspect of the metacarpus remained, but there was no pain on palpation and the horse was sound at walk and trot. Small paddock turnout was instituted at this point. Radiography (a dorsolateral-palmaromedial oblique projection) was repeated every four to eight weeks following hospital discharge. The fourth metacarpal bone underwent significant remodelling over this period. The segment of bone that lacked periosteal new bone formation became demineralised resulting in a wide apparent fracture line before undergoing healing with incomplete bridging bone callus evident in the final set of radiographs obtained on day 316 (Figure 1, 2).

Discussion

This study describes the successful treatment of diffuse Clostridial osteomyelitis (Stage 4A) affecting MCIV with aggressive loco-regional and systemic antimicrobial therapy. This case report also demonstrates the superior diagnostic capability of CT over radiography and ultrasound for the identification of diffuse osteomyelitis of MCIV and unicortical fracture of MCIII. Follow-up radiography performed regularly throughout and after treatment (10 months) demonstrated the evolution of bone healing in this case (Figure 1, 2). At the time of submission of this report (1-year post-injury) the horse is undergoing a normal turnout regimen and is sound. Successful treatment of this type of lesion, in conjunction with a unicortical fracture of MCIII has not been reported previously.

Osteomyelitis presents in a variety of ways in horses but is typically more common in foals compared to adults (Sayegh *et al*, 2001). In adult horses osteomyelitis is generally focal, affecting the cortex and medulla of a specific area of bone (Gibbs, 1994). Occasionally, diffuse osteomyelitis is observed in small bones such as the distal sesamoid bone following solar penetration (DeBowes and Yovich, 1989). In humans, osteomyelitis is staged according to the Cierny-Mader classification system, where the anatomical characteristics of the infection and the physiologic characteristics of the host are utilised (Cierny 3rd et al., 2003, Lazzarini et al., 2004). Using the human classification system; we graded this infection as stage 4A, due to the diffuse infection of the medulla and cortex in a physiologically normal individual.

Surgical treatment for stage 4 osteomyelitis in humans involves un-roofing of the bone and intermedullary reaming, along with removal of sequestra and other necrotic bone. Following such radical debridement in humans the bone often requires metallic implants such as external skeletal fixators to maintain structural integrity, and cancellous bone grafting to enhance healing of the bone. Further, it is recommended to cover the debrided bone with muscle transposition and skin grafts (Lazzarini et al., 2004). This approach was considered to be too destabilising in the case described, increasing the risk of fracture of MCIII during recovery from general anaesthesia, but may have resulted in faster radiological healing. Another option would have been to remove MCIV which has been described to treat proximal fractures of MCIV (Baxter et al., 1992). This was not performed in this case due to concerns about recovery with a unicortical MCIII fracture, development of instability of the carpometacarpal joint, and the potential contamination of the carpometacarpal joint from the infected bone.

Aggressive systemic and loco-regional antimicrobial treatment was instigated in this case as a direct result of the CT findings. Biofilm formation is an important factor for bacterial colonisation and survival in wounds involving bone (Orsini, 2017; Gieling et al., 2019). Sub-optimal antibiotic delivery to bones means that achieving therapeutic antibiotic concentrations within such biofilm is limited by patient toxicity (Anwar et al., 1990; Gieling, 2019). Compared to systemic administration, local and regional methods achieve higher antimicrobial concentrations within the wound bed/infected area for prolonged periods without increasing serum antimicrobial levels (Goodrich and Nixon, 2004) and hence the risk of adverse effects (Orsini, 2017). Daily regional perfusion under standing sedation and local anaesthesia (median and ulnar nerve blocks) was tolerated well by this horse without complication. Gentamycin impregnated PMMA beads were used for local antibiotic delivery followed by gauze soaked in metronidazole solution. PMMA has been used extensively as a carrier material for antimicrobials; however, it has been shown that bacterial biofilms can exist on its surface (van de Belt et al., 2001). Newer materials exist for local antibiotic delivery that are biodegradable and therefore don’t require removal but weren’t used in this case for economic reasons (Hart et al., 2013; Gieling et al., 2019). The aggressive loco-regional antimicrobial therapy should have been extended based on the severity of the osteomyelitis (4a) but was discontinued due to the very positive initial response and financial constraints. It is possible that had this course been extended as the severity of the osteomyelitis dictated that the relapse would not have occurred.

Various classes of antimicrobials were used in this case leading to concerns over potential adverse effects such as colitis (Baaverud et al., 1997) and the development of antimicrobial resistance. Quinolones are critically important antimicrobials for human medicine (Angulo et al., 2009). Enrofloxacin was chosen following the ProtectME guidelines (Bowen and Slater, 2012) as a second line antimicrobial after failure of response to tetracyclines. In addition, it was considered prudent to select an antimicrobial with minimal gastrointestinal side-effects as protracted courses of antimicrobials are often required to overcome osteomyelitis (Goodrich and Nixon, 2004; Gieling et al., 2019). Fluoroquinolones have been found to be efficacious in osteomyelitis caused by Gram positive bacteria, by achieving high bone: serum concentrations, by being effective against adhered bacteria and by penetrating white blood cells (Darley and MacGowan, 2004). Metronidazole was chosen due to its activity against anaerobes and good penetration into bone and soft tissues (Cattin et al, 2008). In similar small animal case reports combinations of metronidazole with amoxicillin-clavulanic acid were selected which may work more synergistically together (Cattin et al, 2008). For equine patients additional considerations of adverse effects, availability, cost and practicality of administration also have to be taken into account (Bowen and Slater, 2012). The combination of antimicrobials used in this horse have not been shown to be synergistic and it remains unclear if this infection would have resolved with treatment with enrofloxacin or metronidazole alone. The choice of antimicrobial used for loco-regional treatment was switched following culture of *C. perfringens* based on the principle that B-lactams such as ceftiofur have improved activity over Gram positive bacteria compared to aminoglycosides such as amikacin.

Human patients with stage 3 or 4 osteomyelitis receive antimicrobial therapy for 4-6 weeks after the last major debridement, based on the observation bone takes roughly this long to-vascularise. Cessation of antimicrobial therapy in this case was also based on clinical progression; 4 weeks after discharge the wound was healed and the limb significantly less swollen. Metronidazole was continued due to ongoing osseous changes on follow-up radiographs and was discontinued at 8 weeks when the horse was found to be sound at walk. The use of SAA to guide therapy was not considered in this horse as the initial SAA value, taken during a period of marked clinical disease, was not significantly elevated. This may reflect this individual patient's inability to mount an acute phase protein response (Jacobsen et al, 2004) or that antimicrobial and anti-inflammatory medications being administered at the time of sampling were supressing SAA production (Busk et al 2010; Lindegaard et al 2010; Stack et al 2019)

Numerous attempts to obtain bacterial culture were unsuccessful including obtaining a sample of periosteum from MCIV and the use of enrichment broth. Unfortunately no samples were taken for culture prior to initiation of antimicrobial treatment by the referring veterinarian, reflecting the importance of sampling at the initial visit as this is often the best chance for obtaining useful results. However, a positive bacterial culture was finally obtained, highlighting the importance of repeating culture in cases refractory to treatment. The positive culture coincided with an obvious clinical deterioration; the horse became painful, and the wound began to drain purulent material raising the possibility that *C. perfringens* infection was secondary. Whilst this possibility remains it was clear that once targeted therapy was implemented the horse showed marked and sustained improvement. A potential criticism in this case was not submitting a bone biopsy for histology. Periosteal bone was submitted for culture and direct Gram stain. However, a biopsy consisting of cortical and medullary bone may have provided a definitive diagnosis earlier in the course of treatment had Clostridial bacteria been observed histologically or cultured. The decision not to perform cortical and medullary biopsy was taken after the discovery of the unicortical MCIII fracture and concerns about the site of the biopsy increasing the risk of fracture during recovery.

Clostridium perfringens was the only bacteria isolated in this case and to the authors' knowledge, this is the first reported case of *C. perfringens* associated with equine osteomyelitis. It has, however, been associated with osteomyelitis in small animals (Muir and Johnson, 1992, Cattin et al, 2008). *C. perfringens* is a Gram positive anaerobe, producing multiple disease syndromes in humans and veterinary species, mediated by production of different toxins (Uzal et al., 2010). *C. perfringens* has been implicated in myositis and myonecrosis following intramuscular injections in horses (Adam and Southwood, 2006, Peek et al., 2003) showing similarities to gas gangrene (Flores-Díaz and Alape-Girón, 2003). Clostridial species are environmentally ubiquitous, likely representing an opportunistic infection in this case.

Typical radiological findings consistent with osteomyelitis include periosteal reaction, focal lysis, loss of trabecular architecture, and new bone deposition but these changes were not apparent initially (Figures 1,2) (Sayegh et al., 2001; Gieling et al., 2019). Serial radiography was useful to show the evolution of osteomyelitis and subsequent healing (Figures 1, 2).

Computed tomography was utilised in addition to conventional imaging modalities in this case (Figure 3, 4, 5). CT allows cross sectional imaging enabling earlier identification of fractures, compared to radiography (Crijns et al., 2014) and has also been shown to be superior for detection of foreign bodies in the distal limb of horses compared to radiography and MRI (Ogden *et al*, 2020) making it an appropriate diagnostic modality in this case. Initially, the unicortical fracture of the third metacarpal bone was not identifiable radiographically, likely due to summation. Osteomyelitis is also more appreciable on CT than radiographs, in part due to the ability to perform multiplanar reconstructions

(Sayegh et al., 2001, Lean et al., 2018). As with radiography, contrast media can increase the value of CT for imaging soft tissues (Puchalski, 2012). Intra-arterial contrast can identify regions with altered vascular permeability that may represent injury or inflammation (Puchalski et al., 2007). Intra-arterial contrast administration was planned for this horse; however, once the diffuse osteomyelitis of MCIV and MCIII fracture were identified it was abandoned. It may have given useful additional insight into potential vascular compromise of the segment of MCIV that later became demineralised. Repeating the CT scan over the course of the treatment would have been very interesting, possibly giving us a better understanding of the progressive changes within bones affected by diffuse osteomyelitis as well as the surrounding soft tissues. However, as changes were so apparent radiographically by this stage, radiography was considered the most practical imaging modality for monitoring the progression of this case. The CT scan findings directly altered the surgical intervention, the recovery method following anaesthesia, post-operative treatment, and enabled detailed aftercare planning with the owner and referring veterinarian. The initial promising clinical results led to premature cessation of aggressive antimicrobial therapy and subsequent relapse.

Magnetic resonance imaging (MRI) was considered as an alternative to CT. MRI can directly image osseous accumulation of fluid, notably on fat-suppressed sequences, and osteolysis, both features of osteomyelitis (Werpy, 2014). Although MRI can be performed on the standing horse, it requires relatively long periods of stillness. Therefore, the young, excitable horse described in this report was considered a poor candidate. As MRI can be performed in the standing horse, repeat scans would have been more practical than CT for monitoring the progression of the pathology.

CT is increasingly available in equine referral practice and has revolutionised imaging of the equine head (Manso-Díaz et al., 2015), and its value in orthopaedics has been gaining attention (Puchalski, 2012) . CT has been shown to be extremely useful both pre- and intraoperatively for orthopaedic cases (Puchalski, 2012), enabling superior surgical planning which may result in reduced surgical time and morbidity (Perrin et al., 2011). The biggest disadvantage of CT for imaging equine limbs is the necessity for general anaesthesia, with considerable risk during the recovery period for catastrophic fracture with any lesion causing bone instability. Fracture risk in this case was mitigated by placement of a full limb cast and performing rope-assisted recovery (Arndt et al., 2020). Additionally, general anaesthesia increases the overall cost of CT, precluding its use in many lameness investigations (and in our case precluding multiple scans to monitor progression). Standing CT systems are now becoming available which will undoubtedly lead to an increase in the use of CT in equine orthopaedics (Riggs, 2019).

Authorship

All authors were involved with the investigation and treatment of the case described. C. Smith, D. Stack and M. Cullen prepared the manuscript. All authors contributed to revision of the manuscript and approved the final version.

Authors declaration of interests

None.

Ethical animal research

Full informed consent for treatment and publication of this report was obtained from the owner of the horse.

Source of funding

None.

Acknowledgements

The authors would like to thank the staff and students of the Philip Leverhulme Equine Hospital who were involved in the care of this case.

Manufacturers' addresses

[1]GE Medical Systems Ltd, Buckinghamshire, UK

[2]Canon Medical Systems, Zoetermeer, Netherlands

[3] Canon Medical Systems, Zoetermeer, Netherlands

[4]Karl Storz, Straβe 34, 78532 Tuttlingen, Germany

[5]Signal blood culture medium, Oxoid LTD., Basingstoke, UK

References

Adam, E. N. and Southwood, L. L. (2006). Surgical and traumatic wound infections, cellulitis and myositis in horses. *Vet. Clin. North Am. Equine Pract.* 22, 335.

Angulo, F. J., Collingnon, P., Powers. J. H., Chiller, T. M., Aidara-Kane, A., and Aarestrup, F. M. (2009). World Health Organisation ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals *Clin. Infect.Dis.* 49, 132-141.

Anon (1991) Guide for Veterinary Service and Judging of Equestrian Events (4th edn.) American Association of Equine practitioners, Kentucky, Lexington, pp 19.

Anwar, H., Dasgupta, M. K., and Costerton, J. W. (1990). Testing the susceptibility of bacteria in biofilms to antibacterial agents. *Antimicrob. Agents Chemother.* 34, 2043 – 2046.

Arndt, S., Hopster, K., Sill, V., Rohn, K. and Kaestner, S. B. R (2020). Comparison between head-tail-rope assisted and unassisted recoveries in healthy horses undergoing general anaesthesia for elective surgeries. *Vet. Surg.* 42(2), 329 – 338.

Bäverud, V., Gustafsson, A., Franklin, A., Lindholm, A. and Gunnarsson, A. (1997). Clostridium difficile associated with acute colitis in mature horses treated with antibiotics. *Equine Vet. J.* 29(4), 279-284.

Baxter, G. M., Allen, D. and Doran, R. E. (1992). Complete excision of a fractured fourth metatarsal bone in eight horses. *Vet. Surg.* 21, 273-278.

Bowen, I. and Slater, J. (2012) Protect ME; The responsible toolkit for equine practitioners

Cattin, I., Liehmann, L., Ammon, P. and Dupre, G., (2008). Subcutaneous abscess caused by Clostridium perfringens and osteomyelitis in a dog. *J. Small Anim. Pract.*, *49*(4), 200-203

Cierny Iii. G., Mader, J. T., and Penninck, J. J., (2003). A clinical staging system for adult osteomyelitis. *Clin. Orthop. Relat. R.* 414, 7-24.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D.,Canali, E. and Leach, M. C. (2014). Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *Plos one.* E92281.

Debowes, R. M. and Yovich, J. V. (1989). Penetrating wounds, abscesses, gravel and bruising of the equine foot. *Vet. Clin. North Am. Equine Pract.* 5, 179-194.

Farr, R. F. and Allisy-Roberts, P. J. (2007). Magnetic resonance imaging. In: Physics for medical imaging. 2nd edn. Elsevier Health Sciences. pp 169-195.

Flores-Diaz, M. and Alape-Giron, A. (2003). Role of Clostridium perfringens phospholipase C in the pathogenesis of gas gangrene. *Toxicon.* 42, 979-986.

Gibbs, C. (1994) Radiological signs of bone infection and neoplasia. *Equine Vet. Educ.* 6, 103-110.

Gielding, F., Peters, S., Erichsen, C., Richards, R. C., Zeiter, S. and Moriarty, T. F. (2019). Bacterial osteomyelitis in veterinary orthopaedics: Pathophysiology, clinical presentation and advances in treatment across multiple species. *Vet. J.* 250, 44-54

Goodrich, L. R. (2006). Osteomyelitis in Horses. *Vet. Clin. North Am. Equine Pract.* 22, 389-417.

Goodrich, L. R. and Nixon, A. J. (2004) Treatment options for osteomyelitis. *Equine Vet. Educ.* 16(5), 267-280

Hart, S. K., Barrett, J. G., Brown, J. A., Papich, M. G., Powers, B. E and Sullins, K. E. (2013). Elution of antimicrobials from a cross-linked dextran gel: In vivo quantification. *Equine Vet. J.* 45(2), 148-153.

Henneke, D. R., Potter, G. D., Kreider, J. L. and Yeates, B. F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4), 371-372

Jacobsen, S., Andersen, P. H., Toelboell, T. and Heegaard, P. M., (2004) Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. *J. Dairy Sci.* 87(10), 3330-3339

Lazzarini, L., mader, J. T. and Calhoun, J. H. (2004). Osteomyelitis in long bones. *J. Bone Joint Surg.* 86, 2305-2318

Lean, N. E., Perkins, N. R. and Ahern, B. J. (2018). Comparison of conventional radiography and computed tomography as aids in the diagnosis of osteomyelitis in 11 foals. *Aust. Vet. J.* 96(7), 257-261.

Long, A., and Nolen-Walston, R., (2020) Equine inflammatory markers in the twenty-first century: a focus on serum amyloid a. *Vet. Clin. North Am. Equine Pract*. 36(1), 147-160

Manso-Díaz, G., García-López, J. M., Taeymans, O. and Maranda, L. (2015). The role of head computed tomography in equine practice. *Equine Vet. Educ.* 27(3), 136-145.

Moor, R. M., Schneiner, R. K., Kowalski, J., Bramlage, L. R., Mecklenburg, L. M. and Kohn, C. W. (1992). Antimicrobial susceptibility of bacteria isolated from 233 horses with musculoskeletal infection during 1979-1989. *Equine Vet. J.* 24(6), 450-456.

Muir, P. and Johnson, K. A. (1992) Anaerobic bacteria isolated from osteomyelitis in dogs and cats. *Vet. Surg.* 21(6), 463-466.

Ogden, N., Milner, P. I., Stack, J. D. and Talbot, A. M. (2020), CT more accurately detects foreign bodies within the equine foot than MRI or digital radiography. *Vet. Radiol. Ultrasound.*

Orsini, J. A. (2017). Update on managing serious wound infections in Horses: wounds involving bone. *J. Equine Vet. Sci.* 55, 123-138.

Peek, S. F., Semrad, S. D. and Perkins, G. A. (2003) Clostridial myonecrosis in horses (37 cases 1985-2000). *Equine Vet. J.* 35(1), 86-92.

Perrin, R. A. R., Launois, M. T., Brogniez, L., Desbrosse, F. G., Vandeweerd, J. M E. F., Clegg, P. D. and Coomer, R. P. C., (2011). The use of computed tomography to assist orthopaedic surgery in 86 horses (2002-2011). *Equine Vet. Educ. 23(6), 306-313.*

Puchalski, S. M. (2012) Advances in equine computed tomography and use of contrast media. *Vet. Clin. North Am. Equine Pract.* 28(3), 563-581

Puchalski, S. M., Galluppo, L. D., Wisner, E. R. and Hornof, W. J. (2007). Intraarterial contrast-enhanced computed tomography of the equine distal extremity. *Vet. Radiol. Ultrasoun. 48(1), 21-29*

Riggs, C. M., (2019) Computed tomography in equine orthopaedics – the next great leap? *Equine Vet. Educ.* 31(3), 151-153

Sayegh, A. I., Sande, R. D., Ragle, C. A., Besser, T. E., Tucker, R. L. and Baker, G. J. (2001). Appendicular osteomyelitis in horses: eitiology pathogenesis and diagnosis. *Compendium.* 23(8), 760

Uzal, F. A., Vidal, J. E., McClane, B. A. and Gurjar, A. A. (2010). Clostridium perfringens toxins involved in mammalian veterinary diseases. *Open toxinology J.* 2, 24

Van De Belt, H., Neut, D., Schenk, W., Van Horn, J. R., Van Der Mei, H. C. and Busscher, H. J. (2001). Staphylococcus aureus biofilm formation on different gentamycin-loaded polymethylmethacrylate bone cements. *Biomaterials.* 22(12), 1670-1611.

Werpy, N. (2014). The use of magnetic resonance imaging for the diagnosis of osteomyelitis. *Equine Vet. Educ. 26(1),* 15-17

Figure Legends

Fig 1: Dorsolateral-palmaromedial oblique views of the proximal metacarpal region of the left fore limb over time. Radiographs are labelled from Day 7 following initial injury to Day 316. Day 7: There is no significant bone change. Day 18 (initial referral): Multifocal perisosteal new bone formation along the abaxial border of the fourth metacarpal bone (MCIV). Day 28: An incomplete curved, slightly indistinct radiolucent line is evident in the palmar lateral cortex of MCIII at the level of the nutrient foramen, consistent with an incomplete uni-cortical fracture. MCIV had a markedly heterogeneous appearance with periosteal new bone formation and multifocal cortical lysis, consistent with osteomyelitis affecting most of the bone's length. Day 95: A transverse, poorly marginated linear radiolucency approximately 3mm in width is evident through MCIV 2cm

proximal to the nutrient foramen at the level of the original wound. The MCIII cortical fracture is no longer apparent but the syndesmosis between MCIII and MCIV is markedly irregular. The architecture of the medulla is disrupted and the palisading periosteal new bone along the abaxial margin of MCIV is coalescing. Day 167: The periosteal bone is coalescing. There is an area of radiolucency extending from the palmaroproximolateral cortex of MCIV into the medulla, in which there are small radio-opaque areas. The latter are likely fragments of necrotic bone. The transverse radiolucency 2cm proximal to the nutrient foramen is evident. Day 316: The abaxial cortex of MCIV and the syndesmosis between MCIII and MCIV are smoothly remodelled and there is some new bone formation in the palmaroproximolateral region of MCIV. An irregularly marginated horizontal radiolucency remains in the mid portion of MCIV.

Fig 2: Dorsopalmar and dorsolateral-palmaromedial oblique radiographs of the proximal metacarpal region obtained on Day 7 and Day 316 after the horse's initial injury. Day 7: There is marked soft tissue swelling affecting the lateral aspect of the limb but there are no abnormalities of the bone. Day 316: There is significant distortion of the size and shape of fourth metacarpal bone (MCIV). There is partial bridging callus around a transverse radiolucent line extending through MCIV 2cm proximal to the nutrient foramen. A radiolucent area extends from the medulla to the palmaroproximolateral aspect of MCIV.

Fig 3: Sequential transverse computed tomographic (CT) images obtained of the left metacarpus from 1cm to 12cm distal to the carpometacarpal joint. The medulla in the proximal part of the fourth metacarpal bone (MCIV) is diffusely hypoattenuating with loss of trabecular bone architecture (2-4cm). The cortical bone on the dorsolateral aspect of MCIV is subtly multifocally hypoattenuating (2cm, 3cm and 5cm). A unicortical, Y-shaped radioluceny consistent with fracture of the palmarolateral aspect of the third metacarpal bone is evident at the level of the nutrient foramen (9cm) and slightly distad (10cm). There is abundant periosteal new bone formation abaxially along MCIV (3-6cm and 8-10cm). The changes effecting MCIV are consistent with diffuse osteomyelitis.

Fig 4: Multiplanar reconstruction of computed tomography images of the left metacarpal region showing a unicortical, Y-shaped fracture of the palmarolateral aspect of the third metacarpal bone at the level of the nutrient foramen. This fracture created a minimally displaced fragment of cortical bone (measuring 27mm x 5mm x 9mm), immediately deep to the articulation with the fourth metacarpal bone and is marked with arrowheads. There is abundant periosteal new bone formation abaxially along MCIV apart for a short (1cm) region, level with the scar of the original wound (ellipse).

Fig 5: Multiplanar reconstruction of computed tomography images showing hypoattenuation throughout the medulla and loss of trabecular bone of the fourth metacarpal bone (black ellipses). There is abundant periosteal new bone formation abaxially along MCIV (arrow heads).