Beyond antibiotics and surgery? Exploring an adjunctive antimicrobial role for MSCs in complex equine orthopaedic infection

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1. Introduction

Equine synovial infections are severe conditions in which septic source control, lavage of the affected synovial cavity, and antimicrobial therapy are essential. When synovial sepsis coexists with septic involvement of osseous structures, the prognosis is generally guarded [1]. In many of such cases, failure to achieve early infection control can result in permanent functional impairment, while progression to osseous involvement further worsens the prognosis, reinforcing the need for prompt and intensive treatment [2]. Nevertheless, in some cases the prognosis for function and even survival may be extremely poor. In this context, mesenchymal stromal cells (MSCs) may represent a valuable potential adjunct therapy. These cells have shown antimicrobial, immunomodulatory, and antibiofilm properties in equine models, although their clinical use as an adjunct in active orthopaedic infections is yet very limited [3-7]. This report describes a case of severe septic tenosynovitis and proximal sesamoid osteomyelitis caused by multidrug-resistant *Enterobacter cloacae* that was managed with a multimodal approach, combining surgery, antibiotics and allogeneic bone marrow-derived mesenchymal stromal cells (BM-MSCs).

2. Case report (Clinical timeline available in Appendix 1)

An 8-year-old Pura Raza Española mare intended for breeding was referred to our hospital as an emergency in 2024 because of an extensive wound on the lateral aspect of the left fore fetlock (Figure 1). Initial radiographs showed no osseous abnormalities (Figure 2). Surgical exploration under inhalational general anaesthesia revealed complete transection of the palmar annular ligament and the palmar digital nerve and vein, with preservation of the palmar digital artery. There was an extensive defect of the digital flexor tendon sheath (DFTS), exposure of the lateral proximal sesamoid bone, and severe injury to the lateral collateral sesamoidean ligament. The flexor tendons initially appeared intact, and the metacarpophalangeal joint did not communicate with the wound on distension testing. Emergency debridement and copious lavage of the DFTS were performed under general anaesthesia (Figure 3a). A second wound was also present at the lateral heel and, after testing, it was found not to communicate with any synovial structure. During the following 3 weeks, repeated DFTS lavage, serial debridement, regional limb perfusions, local and systemic antimicrobial therapy, and support bandaging were performed. At approximately 3 weeks, metacarpophalangeal joint fluid had a TNCC of 0.87 K/µL and glucose of 103 mg/dL, whereas DFTS fluid had a TNCC of 67.73 K/µL, 88.2% neutrophils, and glucose <10 mg/dL, consistent with severe septic tenosynovitis without comparable evidence of sepsis in the joint.

Clinical progress was initially very satisfactory. However, shortly before week 4, pain increased and weight-bearing was reduced, coinciding with radiographic evidence of necrosis and osteolysis of both proximal sesamoid bones - more pronounced laterally (Figures 4a and 4 b). Infection was considered the primary mechanism underlying this finding, although vascular compromise secondary to the trauma may also have contributed [1,2]. Necrotic foci were curetted, and deep bone samples yielded multidrug-resistant *E. cloacae*, which was susceptible only to trimethoprim-sulfamethoxazole among all the antimicrobials initially tested. A single isolate of *Micrococcus luteus* was also recovered from a synovial sample from the fetlock joint. Nevertheless, this agent was not recovered again and was considered of uncertain significance. Tenoscopy was performed to release severe adhesions, debride a deep digital flexor tendon lesion, and reassess sesamoid devitalised foci. Five days later, a sizeable apical fragment of the lateral proximal sesamoid bone was removed during standing wound care (Figures 5a and 5b). Culture of this fragment yielded *E. cloacae* again, confirming persistent osseous infection. The isolate was susceptible to trimethoprim-sulfamethoxazole and, *in vitro*, to fosfomycin, meropenem, and florfenicol, and resistant to aminoglycosides, cephalosporins, fluoroquinolones, and doxycycline.

Initial antimicrobial treatment included systemic penicillin and gentamicin, with regional or local administration of amikacin, gentamicin, and cefazolin. This regime was subsequently modified according to culture and susceptibility results, including trimethoprim-sulfamethoxazole. Source control through lavage and debridement was maintained throughout all the case course. Hoof trimming and balancing were also performed on both the affected and contralateral limbs; the breakover point of the contralateral hoof was set back and frog support material was applied in an attempt to reduce the risk of supporting-limb laminitis. Despite this, pain became extremely severe and difficult to control, requiring multimodal analgesia including NSAIDs, gabapentin, a continuous rate infusion (CRI) of ketamine, opioids, and repeated perineural nerve blocks (combining mepivacaine, dexmedetomidine and corticosteroids). This illustrates the clinical severity, which prompted serious consideration of humane euthanasia.

At this stage, during week 8, orthobiologic treatment with MSCs was added to the therapeutic management. This decision was based on recently published studies and emerging evidence of equine MSC potential antimicrobial effects, including bacteria inhibition and antibiofilm activity. Moreover, this activity has been suggested to be additive or even synergistic with antibiotics [3-5,7,8]. In addition, the ability of MSCs to promote repair in musculoskeletal tissues is well-recognised, which could also contribute to managing this case. A total of fourteen million of allogeneic equine BM-MSCs (passage 4) were administered. The cells were obtained from a clinically monitored donor mare and characterised by trilineage differentiation and surface-marker expression according to previously described protocols routinely used in our laboratory [9]. Two syringes were prepared, each containing 7 million cells in 10 mL lactated Ringer's solution: one was administered into the DFTS and the other intra-articularly into the metacarpophalangeal joint. Although the joint showed no clinical or laboratory evidence of infection, this was considered a prudent measure because of its close proximity and anatomical relationship to the affected structures. No adverse reactions were recorded. Over the following weeks in the frame of the multimodal management programme, weight-bearing, comfort, and lameness progressively improved. At approximately 17 weeks, structural abnormalities persisted, but lameness had markedly decreased. The mare was discharged 18 weeks after admission (Figure 6). We were subsequently informed that the mare had been sold and exported and reportedly had only mild residual lameness. No further clinical follow-up was available.

3. Discussion

This case describes the rarely reported use of allogeneic MSCs as an adjunct in the treatment of chronic septic tenosynovitis with septic osteomyelitis, with a favourable short-term clinical outcome. The chronicity of the infection and/or possible vascular trauma may have contributed to extension of the infection to the proximal sesamoid bones. Proximal sesamoid osteitis/osteomyelitis is uncommon, and concurrent synovial sepsis worsens the prognosis. In the Dabareiner et al. series, three out of eight horses had sepsis, and only one of those three returned to its previous level of activity [1]. In the present case, severe septic tenosynovitis, tendon injury, devitalised tissue, progressive osteolysis, and repeated isolation of a multidrug-resistant bacterium coexisted, illustrating the severity of the condition. This case was managed multimodally, combining MSCs with systemic, intrasynovial, and regional antimicrobial therapy, anti-inflammatory treatment, analgesia, farriery, synovial lavage, and surgical debridement by tenoscopy or conventional surgery. Therefore, the exact contribution of MSCs to the final outcome cannot be determined. However, as suggested by other studies, these cells present several mechanisms that warrant their exploration as an adjunct in a lesion combining active infection, intense inflammation, and synovial, tendinous, and osseous damage. Chronic synovial sepsis may involve fibrin deposition, bacterial aggregates, and biofilm formation, which can reduce antimicrobial efficacy and impair host immune clearance [10,11]. Related to this, MSC-secreted proteases can both inhibit biofilm formation and disrupt mature biofilms, which may help antimicrobials reaching bacteria embedded within biofilm that are otherwise very challenging to eliminate [4,10,11]. In addition, MSCs may exert direct antimicrobial effects through secretion of antimicrobial peptides and indirect effects by enhancing monocyte and neutrophil functions while modulating sepsis-associated inflammation [8,10-11]. Finally, the ability of MSCs to promote repair in musculoskeletal tissues is well-recognised, which could have also contributed managing this case. While equine MSCs from different tissue sources have shown constitutive antibacterial activity [3], this mechanism can be potentiated by cell activation with molecules like TLR ligands [5]. However, this should be regarded as an optimisation strategy under development, rather than a well-established prerequisite [3,5,8]. In this study, non-pre-activated MSCs were used under the precautionary principle. Several of the studies supporting MSC antimicrobial activity have been conducted in vitro, but promising in vivo evidence is also emerging. TLR-activated MSCs combined with antibiotics showed benefits over antibiotic treatment alone in an in vivo equine model of multidrug-resistant *S. aureus* septic arthritis [7] and, more recently, Barton et al. described three horses with chronic or refractory synovial sepsis treated with TLR-activated allogeneic MSCs within multimodal protocols, with resolution of infection, although the specific effect of the cells could not be isolated [10]. In our clinical case, the isolated effects of MSCs in terms of antimicrobial, immunomodulatory, or reparative mechanisms could not be determined. Therefore, their contribution cannot be directly demonstrated or quantified. Nevertheless, local administration of non-preactivated allogeneic BM-MSCs during an active, multidrug-resistant, microbiologically confirmed orthopaedic infection represents a novel clinical application consistent with emerging experimental evidence and for which only a few reports exist so far. The value of this case lies in reporting the potential role of MSCs as adjuncts within a multimodal strategy, never as substitutes for conventional treatment and antimicrobial therapy. Given that antimicrobial resistance is one of the major current public-health challenges, and in line with current One Health approaches, the antibacterial potential of MSCs and its mechanisms of action warrant further investigation, with efforts to apply this strategy to clinical cases that may benefit and to share the outcomes with the veterinary and scientific communities [10,11].

4. Conclusion and clinical relevance

This case highlights the feasibility of incorporating locally administered allogeneic BM-MSCs as an adjunct within a multimodal therapeutic strategy for a severe multidrug-resistant equine orthopaedic infection. No adverse reactions were observed following MSC administration, and progressive clinical improvement ultimately allowed hospital discharge. Although the contribution of MSCs to the outcome cannot be isolated from that of concurrent surgical, antimicrobial and supportive treatments, this case supports further investigation of their potential antimicrobial, immunomodulatory and reparative roles in complex orthopaedic infections.

5. Ethical statement

As all procedures were performed solely for therapeutic purposes, the Ethics Committee of the University of Zaragoza classifies such cases as not subject to ethical review. Written informed consent was obtained for treatment and for the anonymised use of clinical data for teaching and scientific purposes.

6. References

1. Dabareiner RM, Watkins JP, Carter GK, Honnas CM, Eastman T. Osteitis of the axial border of the proximal sesamoid bones in horses: eight cases (1993-1999). J Am Vet Med Assoc. 2001;219(1):82-86. doi:10.2460/javma.2001.219.82.
2. Lawrence CP, Fraser BSL. Septic osteitis of the axial border of the proximal sesamoid bones in two foals. Equine Vet Educ. 2013;25(2):63-66. doi:10.1111/j.2042-3292.2012.00387.x.
3. Cortés-Araya Y, Amilon K, Rink BE, Black G, Lisowski Z, Donadeu FX, et al. Comparison of antibacterial and immunological properties of mesenchymal stem/stromal cells from equine bone marrow, endometrium, and adipose tissue. Stem Cells Dev. 2018;27(21):1518-1525. doi:10.1089/scd.2017.0241.
4. Marx C, Gardner S, Harman RM, Van de Walle GR. The mesenchymal stromal cell secretome impairs methicillin-resistant Staphylococcus aureus biofilms via cysteine protease activity in the equine model. Stem Cells Transl Med. 2020;9(7):746-757. doi:10.1002/sctm.19-0333.
5. Pezzanite LM, Chow L, Johnson V, Griffenhagen GM, Goodrich L, Dow S. Toll-like receptor activation of equine mesenchymal stromal cells to enhance antibacterial activity and immunomodulatory cytokine secretion. Vet Surg. 2021;50(4):858-871. doi:10.1111/vsu.13628.
6. Khatibzadeh SM, Dahlgren LA, Caswell CC, Ducker WA, Werre SR, Bogers SH. Equine bone marrow-derived mesenchymal stromal cells reduce established S. aureus and E. coli biofilm matrix in vitro. PLoS One. 2024;19(10):e0312917. doi:10.1371/journal.pone.0312917.
7. Pezzanite LM, Chow L, Phillips J, Griffenhagen GM, Moore AR, Schaer TP, et al. TLR-activated mesenchymal stromal cell therapy and antibiotics to treat multi-drug resistant Staphylococcal septic arthritis in an equine model. Ann Transl Med. 2022;10(21):1157. doi:10.21037/atm-22-1746.
8. Harman RM, Yang S, He MK, Van de Walle GR. Antimicrobial peptides secreted by equine mesenchymal stromal cells inhibit the growth of bacteria commonly found in skin wounds. Stem Cell Res Ther. 2017;8(1):157. doi:10.1186/s13287-017-0610-6.
9. Cequier A, Romero A, Vázquez FJ, Vitoria A, Bernad E, Fuente S, et al. Equine mesenchymal stem cells influence the proliferative response of lymphocytes: effect of inflammation, differentiation and MHC-compatibility. Animals (Basel). 2022;12(8):984. doi:10.3390/ani12080984.
10. Barton CK, Koch TG, Russell KA, Moorhead R, Mehrpouyan S, Alizadeh AH, et al. Toll-like receptor 3-activated allogeneic umbilical cord blood-derived mesenchymal stromal cells as an adjunct for the treatment of synovial sepsis in 3 horses. J Am Vet Med Assoc. 2026:1-9. doi:10.2460/javma.25.12.0821.
11. Chen S, Jia Y, Yang L, Xie Y, Zhu Y, Li J. Antimicrobial effects of equine regenerative medicine products: a systematic review and meta-analysis of in vitro and in vivo studies. Equine Vet J. 2026. doi:10.1002/evj.70297.

APPENDICES

Appendix 1. Clinical timeline

Time point	Finding / intervention	Relevance
Week 0	Lateral fetlock trauma; radiographs without osseous lesions; extensive injury to the DFTS and palmar structures. Emergency debridement and lavage.	Severe initial injury with synovial contamination.
Weeks 0-3	Repeated lavage, debridement, regional limb perfusions, local/systemic antimicrobials, and support.	Persistent septic process despite intensive treatment.
Week 3	DFTS: TNCC 67.73 K/µL, 88.2% neutrophils, glucose <10 mg/dL. Joint: TNCC 0.87 K/µL, glucose 103 mg/dL.	Severe septic tenosynovitis without comparable joint inflammation.
Week 4	Increasing pain, reduced weight-bearing, and development of sesamoid osteolysis/necrosis. Curettage. Bone culture: multidrug-resistant *E. cloacae*.	Progression to osseous infection; first confirmed deep isolate.
Pre-MSC period	Severe, difficult-to-control pain; advanced systemic analgesia and perineural techniques. Humane euthanasia was seriously considered.	Indicator of extreme clinical severity.
Week 6	Tenoscopy: adhesion release, debridement of the deep digital flexor tendon lesion, and sesamoid reassessment.	Surgical source control and management of tendon damage.
Week 7	Removal of an apical fragment of the lateral sesamoid. Repeat culture: *E. cloacae*.	Confirmation of persistent osseous infection.
Week 8	14 million passage-4 allogeneic BM-MSCs: 7 M intra-articular + 7 M into the DFTS.	Adjunctive cell therapy; no adverse reaction.
Week 17	Marked reduction in lameness despite persistent structural abnormalities.	Progressive clinical improvement.
Week 18	Hospital discharge.	Clinical outcome compatible with management outside the hospital.

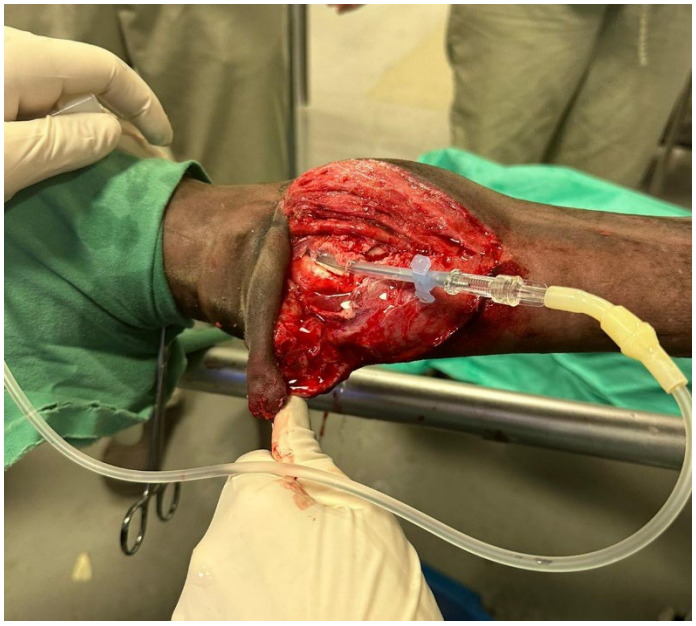
Appendix 2. Figures



Figure 1. Initial appearance of the extensive wound on the lateral aspect of the left fore fetlock at admission.



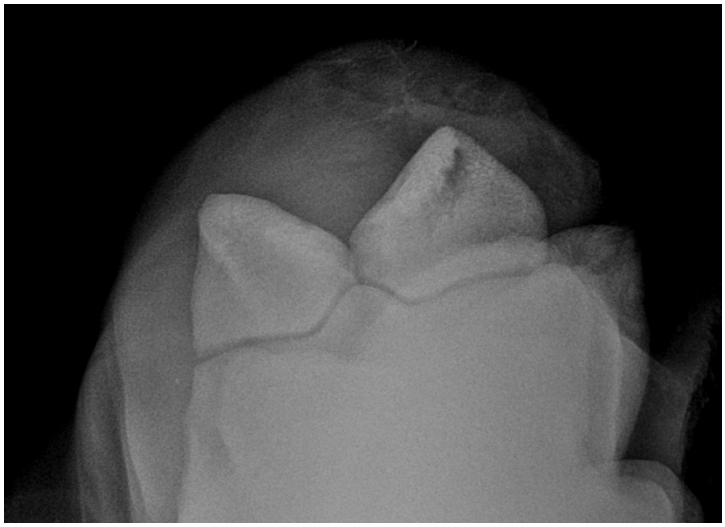
Figure 2. Initial radiograph of the left fore fetlock at admission, showing no detectable osseous abnormalities.





Figures 3a and 3b. Early surgical management of the extensive lateral fetlock wound and associated synovial injury.





Figures 4a and 4b. Radiographs obtained after clinical deterioration, showing osteolysis and fragmentation of the proximal sesamoid bones, more severe in the lateral proximal sesamoid bone, consistent with septic osteomyelitis



Figures 5a and 5b. Radiographs obtained before (a) and after (b) removal of the sizeable apical fragment of the lateral proximal sesamoid bone.



Figure 6. Clinical appearance of the lateral fetlock wound some days before hospital discharge, showing marked soft-tissue healing