Natural vs Experimental Contamination of External Fixator Pins in a Sheep Model

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Introduction: Orthopedic-related infections (ORIs) cause significant economic impact and remain the leading cause of revision procedures following orthopedic implant placement in both humans and animals. Currently accepted models for evaluating ORIs include using abnormally high bacterial inoculation levels and lab-grown bacterial strains[1]. It is well known that even with proper surgical technique and postoperative management of external fixator pins, there is a high rate of pin tract infection, with a range of 45-71%[2,3] for localized infections with up to 4% developing osteomyelitis[2]. The pin site infection rate remains consistent across various postoperative pin care practices and is higher in periarticular pins or pins placed through larger soft tissue envelopes. Establishing a consistent model of a naturally occurring ORI can enhance the evaluation of both local and systemic immune responses to ORIs, as well as assess the effectiveness of proposed treatments. **Methods:** *Animal Model:* In this pilot study, four healthy, 2-year-old castrated male Dorset sheep were divided into two groups: Cohort 1 (natural infection, n = 2) and Cohort 2 (*S. aureus* inoculation, n = 2). Each animal underwent a mid-shaft tibial osteotomy stabilized with a half-pin monoplanar external fixator construct (2 pins proximal and 2 pins distal) without perioperative antibiotics. Cohort 2 was iatrogenically inoculated with 0.2 mL of *S. aureus* at 2.5×10^6 CFU/mL at each of the pin sites at the conclusion of the surgical procedure. Clinical monitoring involved daily exams and weight-bearing assessments. Pin sites were evaluated twice weekly for pain and sensitivity, swelling, heat, and redness, with results combined into daily scores. Radiographs, graded blindly for cortical lucency and periosteal reaction, were taken at scheduled intervals. Cohort 2 was euthanized at 4 weeks, Cohort 1 at 8 weeks, for postmortem analysis, including pin site histology and micro-CT **(Figure 1)**. *Inoculum Preparation:* *S. aureus* (ATCC 25923) was grown overnight in tryptic soy broth at 36.5°C with agitation (235 rpm). A 15 mL aliquot was centrifuged at 1200×g for 10 minutes; the supernatant was discarded. The pellet was resuspended in 10 mL sterile 1X Phosphate-Buffered Saline (PBS) by vortexing and diluted tenfold in PBS before Optical Density (OD) measurement at 600 nm. The bacteria were further diluted to an OD of 0.3, then subjected to three serial tenfold dilutions to reach 2.5×10^6 CFU/mL. Inoculum concentration was confirmed via colony counting. *Pathogen Identification:* Pins were sampled at post-op and during necropsy using aseptic technique **(Figure 1 B1&B2)**. At the time of sacrifice, microbial samples were taken from all pin sites and the tibial medullary cavity and immersed in 1 mL PBS, inoculated onto tryptic soy blood agar, and incubated overnight at 36.5°C. Pins were washed three times by vortexing in sterile PBS, sonicated at 40 kHz and 37°C for 10 min, and streaked onto blood agar. Selected colonies were subcultured on mannitol salt agar. **Results:** One sheep from Cohort 1 was euthanized at 2 weeks due to pin loosening and frame failure, leaving three sheep for analysis. Pin evaluation scores varied in severity and onset between cohorts. Radiographs **(Figure 2)** showed progressive bone changes consistent with septic pin tract infection in both groups. Radiographic scores were higher in Cohort 2 (iatrogenic infection) at 1 week, while Cohort 1 (natural infection) showed minimal changes initially but reached higher scores by 4-7 weeks compared to Cohort 2 at 4 weeks. Postmortem findings included mucopurulent discharge from all pin sites, notably copious at proximal sites in both cohorts. In Cohort 1, all pins were loose, and *S. aureus* was isolated from the pin sites. In Cohort 2, one sheep had all four pins loosened; the other sheep had only the proximal first and second pins loosened. On blood agar, all pins from Cohort 2 cultured positive for β-hemolysis, while 56% of pins from Cohort 1 were β-hemolysis positive. Subculture on mannitol salt agar revealed mannitol-fermenting bacteria on 38% of pins in Cohort 2 and 68% in Cohort 1. Overall, this indicates *S. aureus* was present in all pin site infections, with approximately 20% of pin site infections in Cohort 1 potentially co-infected with *S. epidermidis* **(Table 1)**. Remarkably, Cohort 1 showed signs of infection within 1 month, earlier than the 49-day average reported in our previous study involving a sheep cohort of 35 animals that received intravenous amikacin throughout the 18-week study period of a critical segmental defect fracture healing study stabilized with a uniplanar skeletal fixation frame[4]. **Discussion:** Both Cohort 1 and Cohort 2 exhibited clinically relevant features of pin site infection in sheep, although disease progression and severity differed between the cohorts. Cohort 1 experienced *S. aureus* infection 19 days earlier than in our previous study, highlighting the importance of experimental design in deciding whether to include systemic antimicrobial therapy when modeling clinical pin site infections. The high reproducible rate of pin tract infections is consistent with previous reports in human patients with tibia fractures stabilized with external skeletal fixation[5]. The risk of intramedullary infection of orthopedic hardware increases substantially following pin tract infection[6,7]. We observed all infections at the proximal pin sites with greater soft tissue coverage. The cranial tibialis muscle movement at the proximal pin sites contributes to a soft tissue-pin interface in constant motion. **Significance**: Our findings present the opportunity for a potential large animal model to study naturally occurring ORI, which can be used in future research to evaluate new technologies for reducing implant-related infections. A naturally occurring implant infection model removes the need for supraphysiological inoculum levels that are not reflective of clinical reality and reduces the burden on the investigative team. **References:** [1] Moriarty FT, et al. JOR. 2019 Feb. 37(2):271-287. [2] Kazmers NH, et al. Strat Traum Limb Recon. 2016. 11:75-85. [3] Sims M, et al. J Ortho Nursing. 2000 Aug; 4(2):59-63. [4] Boyes M, et al. VetSurg. 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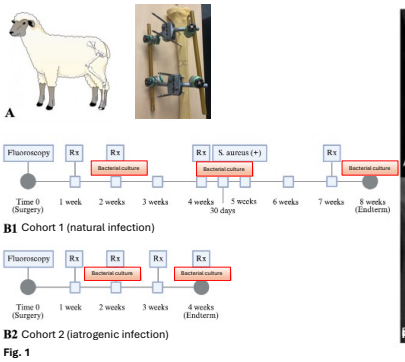

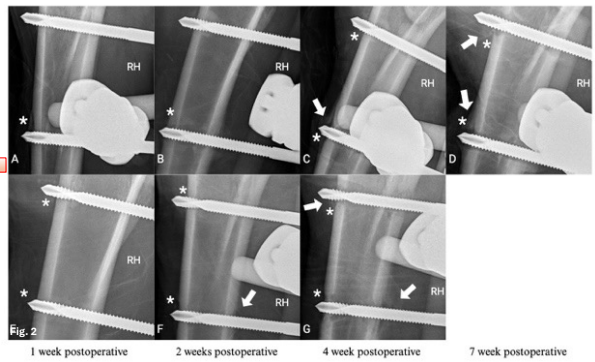

Table 1: Pin Site Microbial Culture Results

	Cohort1	*Cohort2*
Hemolysis		
α	20%	0%
β	56%	100%
γ	24%	0%
Mannitol Fermentation		
-	32%	63%
+	68%	38%
Organism		
Indeterminate	12%	0%
S. aureus	68%	100%
S. epidermidis	20%	0%

Figure 1. A: Diagram of tibial osteotomy model with half-pin external fixator box construct. **B1:** Cohort 1 imaging and infection confirmation (30 days postoperatively) timeline. Study duration was 8 weeks postoperatively. **B2:** Cohort 2 imaging timeline. Study duration was 4 weeks postoperatively. **Figure 2.** Caudocranial radiographs of the proximal first and second tibial pin sites at four timepoints: 1, 2, and 4 weeks postoperatively for both cohorts, and 7 weeks postoperatively for Cohort 1 only. **A–D:** Cohort 1. **E–G:** Cohort 2. Asterisks indicate cortical lucency; arrows indicate periosteal reaction around pin sites. **Table 1:** Infection in external fixator pins with reported incidences between 3% and 80% occurs when planktonic bacteria adhere to external fixator pins and subsequently produce a biofilm, which protects the bacteria from host defences. The most commonly implicated organisms are *Staphylococcus aureus* and *Staphylococcus epidermidis.* Both of these clinically relevant organisms were recovered from pin sites of animals in the naturally occurring infection cohort.