B4: "What is(are) the best preclinical model(s) of orthopedic infection for the evaluation of diagnostic technologies?"

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RESPONSE/RECOMMENDATION: As with all research on living organisms, the "best" model is primarily determined by the hypothesis to be tested, and general animal welfare rules apply (e.g. molecular/mechanistic and initial diagnostic index studies should be tested in small species, while clinically relevant hypotheses on human specific diagnostics might be appropriate for testing in larger or humanized species). With regard to animal models, the following should be declared in all studies, the translational potential ,ethical approval, model design, statistical analyses with prospective threshold of success and failure. Currently, the industry standard for evaluating diagnostic potential is a receiver operating characteristic (ROC) curve analysis of the new technology vs. clinical cultures, clinical signs and symptoms or other FDA approved diagnostic of orthopedic infection. Potential researchers are directed to: *PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) , https://norecopa.no/PREPARE*. Guidelines such as this should be mandated by publishing media. Studies should use standardised innocula of bacteria. Endpoints should be valid and diagnosis should involve quantative microbiological, radiological, serological as well as clinical observations. Newer diagnostic technologies can be correlated with the above modalities and should specifically aim to replicate accuracy and reduce invasiveness. Although validated in silico and in vitro models to assess diagnostics of orthopaedic infection do not currently exist, these technologies are rapidly emerging and may need to be considered in the near future

Level Of Evidence: Expert Opinion

Delegate Vote:

Rationale: A Systematic literature review of PubMed and Ovid Embase databases identified 628 papers relevant to the question. A total of 38 papers were selected for extraction and review through Covidence. Murine and rodent models have been most commonly used in recent years due to advantages in cost, reduced animal welfare concerns, and advancements in the availability of novel diagnostic modalities. They have been termed "first pass" research tools in experiments before advancing to larger models[1]. Large animal models have been developed for specific clinical scenarios such as lower inoculums, and have included both rabbit and pig models of orthopaedic infection, indicating their use in modelling indolent or early infection[2; 3].In light of the elaborate and sophisticated immune environment in humans many would argue the utility of larger animal models The rabbit model allows a good transition to larger groups and shares features of each. The New Zealand White seems to be the commonest breed representing almost 99% of all infection studies[4]. From a mechanistic point of view, pig models may be preferred over murine models due to the closer similarity of the porcine immune system to the human one: pigs have high numbers of neutrophils in the peripheral circulation (50–70%)[3; 5] and have more similar toll-like receptors. In addition porcine bone has similar homeostasis to humans.[3] We have collated the more robust animal models in Table 1.

Diagnostics
Clinical observations are important and researchers are directed to aforementioned guidelines. In addition to normal observations of test animals, measuring gait, weight-bearing symmetry, von Frey testing, toe splaying allow a more comprehensive picture of animal welfare[6; 7].

Quantitative microbiological assessments
Researchers should be cognisant of species specific bacterial strains and know that human derived bacterial samples may not mirror animal models[5; 8]. There needs to be concordance in the mechanism and dosages of inoculation as well as in antibiotic administration if required. How the samples are taken, whether direct culture, sonication or homogenisation of samples is not uniform across studies. Whilst implant related infection is predominantly biofilm based many studies will not pick up planktonic strains and what are termed viable but not-culturable VBNC state. The addition of PCR and Random Amplified Polymorphic DNA (RAPD) analysis of bacterial DNA allows more accuracy. Fluorescent in situ hybridisation (FISH) is a diagnostic tool that looks at ribosomal RNA in biofilm species both sessile and planktonic[9; 10].

Nucleic acid, serology, and protein analyses
Several authors investigated infected models and charted the immune response with PCR[11; 12]. Next generation DNA sequencing has also been studied in preclinical models[13]. The study of protein and gene expression from the serology of animals exposed to bacterial species as compared to traditional culture techniques[14]. Multiplex-Luminnex assays have become popular to study large arrays of serum cytokines, chemokines and antibodies[15; 16]. Rochford et al in a robust model set standards regarding serological levels of immune markers TNF and other interleukins in a mouse study comparing infected and aseptic union[17]. However not all antibodies as detected to bacterial antigens in animals accurately mirror that of the human response as reported in a guinea pig model by Sadovskaya et al[18]. To address this, humanized murine models have been developed to study bone infection[19].

Imaging
X-rays changes of infection have been well documented in a rabbit tibia model[20]. The usage of FDG PET is superior to MRI in a mouse model and correlates well with traditional clinical inflammatory and culture techniques[21]. Odekerken et al reported on a tibial infected NZW rabbit model allowing differentiation between septic and aseptic cases using 18 F-FGD micro PET imaging[22-24]. These studies were corroborated in a rat using a second radiotracer[25]. Wang further studied other radiotracers in the rabbit[26]. Radiolabeled monoclonal antibodies specific to the teichoic acid in bacterial cell wall is also a potential diagnostic tool with PET scanning and has been studied in mice comparing it to bioluminescence and conventional techniques[27]. This is further expanded using indium labelled antibodies and SPECT/CT in a mouse model[28]. Other radiotracers such as 99mTc-ciprofloxacin have shown poor specificity in rabbit models[29]. Micro CT in a mouse and rabbit model favours well when correlated with histological and bioluminescent models[30-32].

Bioluminescence, fluorescence and photoacoustic diagnosis
Bioluminescence imaging (BLI) detects signals emitted by metabolically active luciferase-expressing cells through the tissues of living animals, eliminating the need to euthanize subjects for bacterial quantification[33]. BLI has been used to study bone infection in mice[11], rats[34], and rabbits[35]. BLI is a biomarker of in vivo planktonic growth of bacteria[12], and correlates well with uCT findings in a model developed by Bernthal et al[30]. Ibrahim

subsequently published on an uncemented rodent model exploring bioluminescence and correlating with uCT, MRI and SEM[7]. Niska looked at a genetically modified mouse with a ‘gene knock-in’ allowing bioluminescent and fluorescent optical imaging of bacterial burden by green fluorescent neutrophils[36]. Numerous studies replicate the above models for this diagnostic modality[30; 37; 38]. Targeted fluorescent imaging (TFLI) may allow pathogen specific detection using non-invasive fluorescence imaging with bacterial-specific probes[39]. Mouse models are used in intravital microscopy to monitor infection in real time, longitudinal imaging of bone marrow [LIMB] has been shown to be feasible in this model while permitting characterization of early bacterial proliferation and immune cell behaviours such as neutrophil swarming within the peri-implant region[38; 40]. Another non-invasive technique is photoacoustic imaging (PAI). The two-dimensional information acquired (up to a depth of 8cm) from ultrasound can be reconstructed into a three-dimensional representations of the underlying tissue and associated signal providing tissue detail comparable to that of conventional MRI[33; 38; 40; 41].

Conclusion

The ambition of many of these diagnostic technologies is to aim for a less invasive mechanism of infection surveillance and hence reduce animal morbidity and numbers required.

Author/Year	Study	Animal	Ethics/Stats	Technique	Diagnosis	Reference No
Xie 2024	Intravital imagingMouse femur plating	Mouse Femoral plating	Yes IACUC/robust stats	MRSA Plating	IVI and CFU	32
Wang 2022	F-FDG Aseptic versus septic	Rabbit NZW femur	Yes ARRIVE/Robust stats	S Aureus/epi and screw direct innoculation CFU	Serology, Micro CT, FDG	20
Hadden 2021	Novel Hemi model	Rat/ femur	Yes	S Aureus innoculation	Photoluminescent, gait, uCT, SEM	37
Fan 2020	Longitudinal study of PJI in Rat knee	Rat/ femur/Tibia	Yes IACUC/robust stats	Implantation poly and metal into knee bones/direct innoculation	Excellent Gait, physical properties coupled with uCT, Histology.	6
Jensen 2017	Assessing innoculum required for IOM in a porcine model	Porcine Tibia	Yes National/Robust stats	Implantation of K wire with innoculum of S Aureus	Serology, CT, bloods, clinical, histology, CFU	3
Carli 2017	Novel Mouse model	Mouse Tibia	Yes Local/Robust stats	Tibial platform in mouse innoculated s aureus	Clinical, serology, CFU, Radiological	38
Wang 2017	Investigaing BLI and PAI	Mouse Femur/Knee	Yes Local/Robust stats	Retrograde femoral k wires with direst innoculation S Aureus	Compares and documents BLI and PAI	35
RochfordETJ 2016	Charting immune response to fracture infection	Mouse Femur	Yes National/Robust stats	Mouse fix plate with innoculum	Serology qPCR, histology, radiological, cytokine.	13
Ren 2012	Looks at utility of PET in diagnosing aseptic and septic loosening in an implant model	Mouse Tibia	Yes IACUC/robust stats	Mouse poly particles and innoculum around implant	Histology, CFU, CT, PET	19
Li 2008	Kinetis of infection serology, BLI.	Mouse Tibia	Yes Local/Robust stats	Pre inoculation of k wire and implantation into tibia	Serology, PCR, uCT, BLI, Histology	11
Ibrahim 2022	New Gram neg model of PJI	Rat/ femur	Yes Local/Robust stats	Innoculation Biofilm P Aeruginosa	CFU, Gait, Sem, MRI,uCT	29

Table 1. Selected animal models for diagnostics

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