ICM 2025 Question B6: "Are bilateral animal models of infected bone defects ethical?"

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RESPONSE/RECOMMENDATION: In jurisdictions where research on infected bilateral bone defects in animal models is permissible, the following should be considered in addition to local Institutional Animal Care and Use Committee (IACUC) approval. 1) Scientific assessments of mobility and functionality that likely go beyond established standards for single limb bone defect infection models as they pertain to animal welfare (need for euthanasia) and pain management (need for analgesic administration) should be included. 2) The cumulative effect of all the defects on animal welfare with correctly applied analgesic treatment and animal management should not exceed the effect of a single infected defect). The overall scientific rigor of the research should provide formal proof-of-concept. 4) Assurances that there is a scientific justification for the use of a bilateral model and it is not being applied primarily for cost-effectiveness reasons. And 5) the research needs to comply with national and international regulations governing animal research, such as the Animal Welfare Act (AWA) in the United States, Directive 2010/63/EU in the European Union, and guidelines from organizations like the National Institutes of Health (NIH) and the World Organization for Animal Health (OIE). There are several other institutions that have published guidelines such as the ARRIVE (Animal Research Reporting of In-vivo Experiments) guidelines and those formulated by the Association for assessment and accreditation of laboratory Animal care. Ultimately, it behoves researchers to at least streamline and gain uniformity in unilateral animal models. Researchers should also strive for better techniques, outcomes, and statistical powering before advancing into the realm of bilateral models.

LEVEL OF EVIDENCE: Expert Opinion

DELEGATE VOTE: Agree: 28/70% vote, Disagree: 0/0%, Abstain: 12/30%

RATIONALE: This question was motivated by recent events in the international orthopaedic research community that challenged the omnipotence of an individual IACUC's ability to approve research on bilateral animal models of infected bone defects based on the following ethical concerns. 1) Ethics on acceptable pain and suffering to be endured by experimental animals varies both between and within countries Thus, to achieve world-wide acceptance of experimental findings, internationally recognized ethical standards need to be used for ethical approval of research on experimental animals. 2) There are no validated infected bilateral injury models that ensure pain and suffering thresholds are not exceeded, and the established outcomes of animal welfare (i.e. lameness) were not designed to assess infected bilateral injuries. 3) IACUCs typically do not contain the scientific expertise to know if primary outcomes of a study will be compromised by gross health consequences of bilateral infected bone defects. The importance of this question also derives from new and evolving restrictions on the use of animal models for research in several countries around the world, which includes prohibitions on the use of species (e.g. dogs and cats) and war-wound models (i.e. multiple severe traumatic infected injuries) that were previously tolerated and published in literature.[1-10] In addition to animal welfare concerns, the scientific rigor of published peer-reviewed preclinical studies of bone infections in large animals and non-rodent models has also been challenged. This is also an ethical issue as most IACUCs are not versed in current technologies that should be used to assess infected bone defects, and thus cannot evaluate the science to make the determination that the research is ethical. These concerns are highlighted by a systematic review of 316 qualifying studies (254 rabbit, 16 pig, 23 dog, 11 goat, and 12 sheep), which reported that less than 5% were adherent to contemporary standards for scientific rigor and

reproducibility.[11] Specifically, the authors found global deficiencies in: 1) methodology, 2) definitions of infection, randomization, and power analyses, and 3) blinding to prevent biased results. Lack of scientific rigor in histology outcomes was cited as a particular concern, as few studies used validated semi-quantitative scoring of the lesions, and several had no objective quantification of outcome. Other deficiencies that have been tolerated in the past but are now required to establish formal proof of concept include: 1) quantitative bacterial burden assessment of the inoculum and in tissue and on implants at prospective endpoints, use of antibiotics and standard of care treatments in control groups, and 3) blinded quantitative analyses of antimicrobial effects and sterility outcomes. Given the critical need for trustworthy musculoskeletal infection animal model research, several groups have published scientific guidelines that include details related to the model (i.e. implant, injury, surgery, treatments, outcomes), pathogen, infected animal, in vivo studies, and post-mortem analyses, all of which are of crucial importance for validation of results and reproducibility.[11; 12]

With respect to the specific issue of bilateral models for infected bone defects, they may indeed reduce the number of animals required for the study, however, there is substantial concern that this will come at the expense of animal welfare. There are also important potential concerns related to spread of bacteria across limbs, and spread of antibacterial treatments across limbs, particularly if the two limbs differ in any experimental parameter (e.g., one is infected and the other not, or one limb receives local treatment and the other not). These latter issues may be resolved through careful study design and control groups.

The development of bilateral animal models of bone infections presents a high risk of mortality due to the potential of bacteraemia, but also occurrences of physiological responses leading to general status impairment, such as severe body temperature changes, inappetence and consequent weight loss. Systemic inflammatory reactions and pain, persistent recumbency may be confused with a lack of lameness and may elude investigators. There is potentially merit in the study of single bone bilateral models i.e. radius/ulna/fibula in limbs with two bones. This would allow 'intra-animal controls'. The investigations and interventions should be within reason, to allow animal comfort rather than in an effort to reduce numbers.

METHODS:

A systematic literature reviews of PubMed and Embase Ovid databases failed to identify any peer reviewed literature that directly addressed the question. On widening our search criteria we discovered 5 studies relating to bilateral animal infected models.

In 1993 Spangnolo et al that looked at a bilateral rat tibia model of osteomyelitis over a 6 month period taking histological radiological and microbiological quantitative studies at time points[13].

A second study examined bilateral rat tibia model and implanted colonized K wire with a secondary procedure to allow photodynamic therapy, bioluminescence was utilized to monitor bioburden. The study was in compliance with Canadian ethical guidelines and animal welfare was reported as satisfactory[14]. Johannson et al in 1991 reported on a bilateral rabbit model of tibial metaphyseal colonisation with Bacteroides fragilis. The second limb served as an uninfected control. After 18 weeks 4 of the 5 models showed infection in the control side hence demonstrating the significant risk of bacteraemia spread across limbs in these models. It is also an enteric pathogen rarely if ever seen in bone infection[15]. Haenle et al in a well-constructed bilateral rat tibial metaphyseal model, which shows robust end points and analysis allowed intra animal controls and examined varying inoculums of *S. aureus*[16].

In a recent canine study, Schweser et al looked at bridging bilateral ulnar defects inoculated with *S. aureus* plates, which was followed by another procedure 3 weeks later where sampling and four interventions were executed after irrigation and debridement[17]. The authors cited previous proof of concept articles in a rabbit unilateral model. The treatments involved antibiotic controls and bacteriophage therapy, and animals were sacrificed after 11 weeks and subjected to biofilm CFU and histological assessment. The study cites a previous non infected bilateral defect canine

model as precedence. It utilizes time point overlaps and 2 operative procedures with an infusion apparatus on one set of animals, this raises the whole animal welfare issue although the author reports normal clinical observations throughout. There is ambiguity regarding whether "in-animal controls" are used with the authors citing ethical approval and reduced numbers, ANOVA statistics are used showing variable results in combined treatments[17].

The paucity of bilateral infected studies may also be suggestive of a publication bias where some journals will not publish such papers as there may be ethical clashes or negative results. However, several studies and agency guidelines on the ethical use of animal models in research were considered in making this recommendation[18-33]. We also identified 13 peer reviewed research articles on uninfected bilateral injury models[33-44], of which only 5 provided information on animal welfare, and 6 reported premature death of study animals (Table 1), further highlight the needs and opportunities for improvement in orthopaedic animal research in general. Moreover, only one of these studies was published after 2018, which may be due to changes in ethical views on these animal models.

CONCLUSION: Whilst there several bilateral models of bone defects reported in the literature there are only 5 to our knowledge reporting upon bilateral infected models.

Authior	Animal Species	N° of animals + (N° of control)	Long bone teste with fixation method	Anaesthesia	Pain management + (Duration)	Walking	Wellbeing	Lameness	death
K. Liu et al. (2013)	C57BL/6 mice	91(18)	Femur (plate and screw fixation)		buprenorphine 2x/day (2 days)	allowed unrestricted movement after anesthesia	Weight monitoring daily for 7 days pre- and post-operation; normal activities such as eating and drinking by the day after surgery	No information	3
A. Chaubey et al. (2013)	Mice (Double transgenic mice, Col1/Col2)	36(9)	Femur (plate and screw fixation)	isoflurane	not further mentioned	After recovery unrestricted activity until sacrifice	No information	No information	0
K. D. Johnson et al. (1996)	Adult mongrel dogs	23	Radius (external fixator)	sodium pentothal	buprenorphine 12 h (1-2 days) and flunixin meglumine daily (1-3 days)	bilateral weight-bearing 8 h post operatively; normal gait within 24 h	No information	1 case ulna fracture	2
K. Lam et al. (2024)	Dogs (specifically, skeletally mature purpose-bred research hounds)	3	Ulnar (plate and screw fixation)	No information	morphine, tramadol after 2nd surgery: morphine (3 days)	unrestricted activity in their individual kennels, after 6 weeks were walked on a leash for 15 min 5 times/wk	No information	No information	0
D. Paterson et al. (1997)	Dog (mixed breed)	69	Tibia (IM rod with external pins)	No information	No information	allowed to befully active for at least eight weeks	No information	No information	19
A. Oryan et al. (2016)	Rat	16	Radius (no fixation)	ketamine and xylazine	No information	No information	plastic cages in a room with ambient temperature of 24°C, allowed ad libitum access to water and laboratory pellets	No information	0
M. D. Zhao et al (2016)	Rabbit (New Zealand white adult)	40	Radius (no fixation)	diazepam and ketamine	buprenorphine	No information	12-hour light/dark cycle, monitored (body temperature, breathing, and feeding behavior). Minimal signs of distress observed in the majority of cases	1 case forelimb fracture Deformity Exclusion	1
J. Xing et al. (2014)	GFP+ transgenic Mouse	36	Femur (plate with wires)	sodium pentobarbita	Buorenorphine hydrochloride every 12 hours (1 day)	allowed free and unrestricted weight-bearing activities without external support	feeding behaviors were unaffected post-surgery	Normal gait after 10 days	2
M. Markel et al. (1991)	Dog (mixed population)	20	Tibia (external fixator)	sodium pentobarbital	No information	allowed to walk freely	No information	No information	0
D. A. Chakkalakal (1999)	Sprague-Dawley Rats	Unknown	Fibula (no fixation)	No information	No information	normal ambulation within 2 days after surgery	No information	No information	unknown
M. Fujita et al. (1999)	Wistar Rats	48	Tibia (IM pin)	No information	No information	normal activity within a few days post operatively	No information	Normal activity	0
T. Balaguer et al. (2018)	Dog (Beagle)	8	Femur (not mentioned)	fentanyl patch 12h before surgery	Meloxicam (5 days) and morphine if necessary	After 3 days allowed to ambulate freely with daily physical examination	pain assessed every 2h during the first 24 h, then every 6 h for the following 5 day	No information	2
X. Chen et al. (2015)	Axolotl (Ambystoma mexicanum)	36(16)	Fibula (no fixation)	No information	Tricaine mesylate (2 hours)	No information	No information	No information	0

Table 1. Information on Animal Welfare in Published Studies with Uninfected Bilateral Injuries.

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