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Outcome of nonunion fractures in dogs treated with fixation, compression resistant matrix, and recombinant human bone morphogenetic protein-2

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Keywords

Bone regeneration, bone morphogenetic protein 2, BMP-2, compression resistant matrix, nonunion

Summary

Objectives: To report the use of compression resistant matrix (CRM) infused with recombinant human bone morphogenetic protein (rhBMP-2) prospectively in the healing of nonunion long-bone fractures in dogs.

Methods: A longitudinal cohort of dogs that were presented with nonunion fractures were classified and treated with CRM soaked with rhBMP-2 and fracture fixation. They were followed with serial radiographs and evaluated for healing times and complications according to the time frame and definitions previously established for orthopaedic clinical cases.

Results: Eleven nonunion fractures in nine dogs were included. Median healing time was 10 weeks (range: 7–20 weeks). Major perioperative complications due to bandage morbidity were encountered in two of 11 limbs and resolved. All other complications were minor. They occurred perioperatively in eight of 11 limbs. Minor follow-up complications included short-term in one of two limbs, mid-term in one of three, and long-term in four of five limbs. Nine limbs returned to full function and two limbs returned to acceptable function at the last follow-up.

Clinical significance: Nonunion fractures given a poor prognosis via standard-of-care treatment were successfully repaired using CRM with rhBMP-2 accompanying fixation. These dogs, previously at high risk of failure, returned to full or acceptable function.

categories (3, 4). Differentiation between oligotrophic viable and atrophic nonviable nonunions via radiography alone is debatable. Ultrasonography and histological assessment of vascularity are required to differentiate between these categories (5). Despite routine treatment with fracture fixation and autogenous or allogenic bone graft, nonunion treatments can fail.

Bone morphogenetic proteins are multifunctional growth factors within the transforming growth factor beta super family (6). They are endogenous signalling peptides, stimulating a series of events leading to differentiation of pluripotent mesenchymal stem cells into osteoprogenitor cells, osteoblasts, and ultimately osteocytes, resulting in new bone formation (7–9). Use of bone morphogenetic protein has been successful for bone repair (6). Currently, two recombinant human bone morphogenetic proteins (rhBMP-2 and rhBMP-7) are FDA^a approved for use in specific medical conditions in limited human populations including maxillofacial, spinal fusion, open tibial fracture, as well as compassionate use in long-bone nonunions (10–14).

The successful use of rhBMP-2 requires a delivery vehicle, and is dependent on quantity, concentration, and time of application (15–17). An ideal carrier optimizes predictable bone formation through compatibility and bioresorbability while, particularly in a gap model, maintaining structural integrity (18). A carrier suitable to all applications has not been identified, however numerous carriers have been met with success, including inorganic materials (calcium phosphate ceramics), natural

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Introduction

Nonunion after long-bone fracture repair in dogs represents a potentially devastating complication. Rates of nonunion compli-

cations in veterinary medicine are unknown, however, reported rates of delayed or nonunion in humans can be up to 10% (1, 2). Causes for nonunion are reported and classified into viable and nonviable

polymers (collagen), synthetic polymers (polyglycolic acid), and composites (19–22). Compression resistant matrix (CRM) is a collagen sponge with embedded granules of hydroxyapatite and tricalcium phosphate, allowing a semi-rigid structure with resistance to compressive forces *in vivo* and has been shown to be appropriate for defect sites in multiple studies (18, 23–26).

Previous successful studies using rhBMP-2 delivered in a CRM in dogs are limited to the mandible, including reconstruction after mandibulectomy and nonunion mandibular fractures (23–24, 26). A single case of nonunion of the distal radius in a Pomeranian was reported as part of a literature review (27).

The purpose of this prospective longitudinal cohort study was to report the use of CRM infused with rhBMP-2 in the healing of nonunion long-bone fractures in dogs. Our hypothesis was that all fractures

treated with fixation and CRM with rhBMP-2 would achieve clinical union.

Materials and methods

Cases

All dogs that were presented to the Veterinary Medical Teaching Hospital and the University of California-Davis for nonunion fractures with failure of at least one previous surgery were recruited for this study. A minimum of two radiographic views of the affected bone were obtained with a standard calibration device for magnification. Dogs with any radiographic evidence of bone callus were excluded. Informed consent to use rhBMP-2 off label was obtained from all clients. Two authors used the Weber-Cech terminology and categorized the cases, as either defect, dystrophic, necrotic, or oligotrophic-atrophic nonunion and were labelled as infected if

this was suspected due to either radiographic signs or appearance at surgery (4). Historic data were collected from owner history and medical records (► Table 1).

CRM and rhBMP-2 preparation

This study utilized CRM^b and rhBMP-2^c. The volume of the defect was measured in three dimensions after surgical fixation, and a fitted section of CRM with a length 2 mm greater than the defect was cut. The prepared CRM was soaked with 0.5 mg/mL rhBMP-2 at a volume corresponding to 50% of the CRM, 10 minutes prior to implantation.

a FDA: U.S. Food and Drug Administration, <http://www.fda.gov/default.htm>

b MasterGraft Matrix[®]: Medtronic, Memphis, TN, USA

c rhBMP-2: Pfizer, Cambridge, MA, USA

Table 1 Signalment, fracture classification, and previous treatment.

Patient	Breed	Weight (kg)	Age (years)	Affected bone	Nonunion classification	Time from initial fracture to rhBMP-2 (months)	Previous surgeries	Previous fixation
1	Italian Greyhound	3.0	3	R radius/ulna	Defect	16	5	1.5/2.0 mm 15-hole VCP
1	Italian Greyhound	3.0	3	L radius/ulna	Dystrophic	10	1	1.5/2.0 mm 10-hole VCP
2	Rat Terrier	7.1	1	R tibia	Atrophic-Oligotrophic	3	1	Type II ESF and osteoallograft
3	Golden Retriever	24.3	12	L tibia	Dystrophic	9	3	IM pin + cerclage; 10-round-hole plate (unknown)
4	English Pointer	29.7	11	R lateral humeral condyle	Atrophic-Oligotrophic	4.5	1	5.5 mm cortical lag screw + 2.4 mm 8-hole LCP + cancellous graft
5	Schipperke	7.1	2	R radius/ulna	Atrophic-Oligotrophic	6	2	Ring ESF distraction
6	Border Collie	16.0	9	L tibia	Atrophic-Oligotrophic	5	1	ESF hybrid MIO + tobramycin Osteoset beads
7	Cocker Spaniel	12.0	10	L lateral humeral condyle	Atrophic-Oligotrophic	~48	5	Multiple condylar screws and pins (unknown)
8	Miniature Poodle	3.0	2	R radius/ulna	Dystrophic	2	1	1.5 mm 8-hole condylar LCP
8	Miniature Poodle	3.0	2	L radius/ulna	Dystrophic	2	1	1.5 mm 12-hole adaptation plate
9	Mixed breed	12.0	4	R radius/ulna	Dystrophic	3	1	9-hole LCP (radius), 7-hole LCP

ESF = external skeletal fixation; IM = intramedullary; LCP = locking compression place; L = left; R = right; MIO = minimally invasive osteosynthesis; rhBMP-2 = recombinant human bone morphogenetic protein 2; wk = week; VCP = Veterinary Cuttable Plate.

Table 2

Intra- and post-operative data, encompassing method of fixation with recombinant human bone morphogenetic protein 2 (rhBMP-2) and compression resistant matrix (CRM), defect size, evidence and treatment for osteomyelitis, and post-operative care and follow-up.

Patient	Fixation with rhBMP-2	Defect size (mm)	Evidence of osteomyelitis	Antibiotic beads at surgery	Postoperative antibiotics	External coaptation	Time to healing (weeks)	Follow-up (months)
1	2.0 mm 14-hole LCP	65	No	-	-	Soft padded (2 wk)	9	24
1	2.0 mm 14-hole LCP	2.5	No	-	-	-	10	24
2	2.0 mm 13-hole LCP	32	No with culture	+	-	-	10	2.5
3	3.5 mm 10-hole LCP	16	Yes with culture	+	+ (6 wk)	-	10	24
4	Same as previous/ not removed	1	No	-	-	-	16	24
5	2.4 mm 10-hole LCP	5	No	-	-	-	6	24
6	Same ESF	10	No	-	+ (2 wk)	-	10	6
7	3.5 cortical transcondylar screw and washer + 2.4 mm 4-hole LCP	1	No with culture	-	-	-	20	12
8	7-hole LC-DCP	2	No with culture	-	-	-	8	12
8	8-hole LC-DCP	2	No with culture	-	-	-	8	12
9	2.4 mm 10-hole LCP	10	No	-	-	Caudal splint (4 wk)	7	4

CRM = compression resistant matrix; ESF = external skeletal fixation; LCP = locking compression plate; LC-DCP = limited contact dynamic compression plate; rhBMP-2 = recombinant human bone morphogenetic protein 2; wk = week.

Surgical technique

All patients underwent general anaesthesia with surgical approach and removal of any unstable previous implants. For diaphyseal fractures, an osteotomy was performed on all fracture edges until bleeding bone was exposed, or until further debridement was prevented by fragment and required implant size, in order to allow a minimum of two screws or positive profile pins to be placed in each segment. The bones were secured with bridging implants, leaving a fracture gap as approximated to the anatomical length of the bone (► Table 2).

The resulting defect was measured to prepare a corresponding CRM infused with rhBMP-2 solution as previously described. The site was lavaged copiously with sterile saline. The CRM was implanted into the defect, ensuring friction with osteotomized bone ends. Instruments in contact with rhBMP-2 were removed from the sterile field and surgeon gloves were changed. Overlying fascia, soft tissues and skin were closed over the implant and CRM with rhBMP-2.

The two fractures of the lateral portion of the humeral condyle were repaired with a transcondylar cortical screw in lag fashion and lateral locking compression plate (LCP). In one fracture with intact and stable implants, a 4.5 mm hole was drilled proximal to the transcondylar screw and filled with CRM with rhBMP-2. The transcondylar screw was tightened and a thin layer of CRM with rhBMP-2 was placed over the cortical surface of the fracture at the lateral metaphysis. A second lateral humeral condylar fracture had breakage and loosening of all implants (multiple screws and pins) placed approximately four years prior to presentation. These implants were surgically removed. The surface of the fracture was debrided and a thin layer of CRM with rhBMP-2 was placed on the fracture surface of the lateral portion of the condyle. The fracture was then stabilized with a 3.5 mm cortical screw in lag fashion and a 2.4 mm LCP plate^d and locking screws^d.

All dogs were treated with intra-operative cefazolin (30 mg/kg [13.6 mg/lb] IV q90–120 minutes). The use of intra-operative culture (taken after lavage and prior

to bone morphogenetic protein implantation), vancomycin-impregnated absorbable antibiotic beads^e, postoperative antibiotic medications, and external coaptation was at the discretion of the surgeon and was recorded. Opioid and non-steroidal anti-inflammatory medications were used for pain control for seven to 12 days post-operatively.

Diagnostic imaging

Radiographs of each limb were obtained immediately postoperatively and at intervals as clinically indicated. Fractures were considered healed when they demonstrated evidence of confluent cortical bone and bridging callus formation on three of the four visible cortices appreciated on orthogonal radiographs, allowing return to normal activity. Determination was dependent on the independent assessment of two board certified specialists (AK, BA), one of whom was blinded to case informa-

^d DePuy Synthes Vet, West Chester, PA, USA

^e Osteoset® Resorbable Mini-Bead Kit: Wright Medical Technology, Inc., Memphis, TN, USA

tion and postoperative time point. The blinded assessor read each film twice. If a discrepancy between readers occurred, the later healing time was chosen as the data point.

Clinical evaluation

All complications were recorded according to the previously published definitions and criteria for subjective clinical study results (28). Complications were defined as minor, major, or catastrophic. Time frame and definitions of evaluations and complications were followed in the orthopaedic definitions as previously outlined (28).

Results

Nine client-owned dogs with 11 fractures determined to be nonunion were included in this study. Median age was three years (range: 1 – 12 years). Breeds included Italian Greyhound (n = 1, bilateral fractures), Miniature Poodle (n = 1, bilateral fractures), Golden Retriever (n = 1), English Pointer (n = 1), Schipperke (n = 1), Border Collie (n = 1), Cocker Spaniel (n = 1), Rat Terrier (n = 1), and mixed breed (n = 1). Included fractures were radiographically categorized as dystrophic nonunion (n = 5), oligotrophic-atrophic nonunion (n = 5), or defect nonunion (n = 1). Upon surgical approach to the nonunion, all fractures contained fibrous soft tissue with lack of callus formation, and no haemorrhage was encountered on initial debridement. Upon osteotomy, bleeding bone was eventually encountered. All cases had nonunion from implant placement or surgical technique from one or multiple open surgical procedures or a combination of both. Other contributing reasons for nonunion included implant failure (8/11 fractures), critical defect size (2/11), radiation necrosis secondary to soft tissue sarcoma removal (1/11), and incomplete intracondylar ossification (1/11). Each dog had undergone at least one previous surgical procedure (median: 1; range: 1–5). The median time from initial injury to inclusion in the study was five months (range: 2 – 48 months) (► Table 1). Debrided defect size median

was 2.5 mm (range: 1 – 65 mm) (► Table 2).

Five fractures were cultured due to suspicion of osteomyelitis, of which one cultured positive for small numbers of *Staphylococcus pseudintermedius* and *Staphylococcus intermedius*. Two patients had vancomycin-infused resorbable mini-beads^e placed after insertion of rhBMP-2 infused CRM at the time of the nonunion surgical procedure. One of these two cases had a positive intra-operative culture. Two different cases had been treated with antibiotic beads during a previous surgery, prior to nonunion treatment with rhBMP-2 infused CRM.

Two dogs were maintained on antibiotic medication postoperatively (amoxicillin with clavulanic acid); one dog was treated for two weeks because of it having an open fracture initially. One dog was treated for six weeks because of confirmed infection. Two dogs with radial and ulnar fractures had external coaptation applied postoperatively; one had a soft padded bandage for two weeks and the other a caudal splint for four weeks.

Clinical evaluation

Major perioperative (<3 months) complications were encountered with two of the 11 limbs and were associated with bandage morbidity requiring anti-inflammatory and pain medications at two weeks postoperatively. These resolved completely. Minor reported perioperative complications were noted in eight of the 11 limbs and included swelling of the incision site (n = 3), mild serosanguinous discharge (n = 1), weight bearing lameness (n = 3), decreased range of motion of the carpal joint (n = 3), decreased range of motion of the elbow joint (n = 2), and periodic conscious proprioceptive deficits (n = 1). The incisional swelling and discharge minor complications resolved within eight weeks.

Follow-up on all dogs was 2.5 to 24 months (median: 12 months).

One dog had only perioperative (0–3 months) follow-up and this dog had occasional proprioceptive deficits when standing at 2.5 months. The owner was a veterinarian and reported that the deficits completely resolved.

Two dogs had only short-term follow-up (3–6 months) and the reported minor complication of decreased range of motion of the carpus persisted in one dog.

Two dogs were last evaluated at mid-term (>6–12 months) and out of three limbs, one minor complication was reported (continued decreased range of motion of the elbow joint).

Four dogs (5 limbs) were evaluated long-term (>12 months). All long-term complications were minor and included mild carpal valgus with a decreased range of motion (n = 1), mild tarsal valgus (n = 1), mild continued lameness with a decreased range of motion of the carpus (n = 1), and a decreased range of motion of the elbow (n = 1).

Nine out of 11 nonunion limbs returned to full function postoperatively, and the other two patients had acceptable function as previously defined (28). One patient, which was followed for 24 months, exhibited decreased range of motion of the carpus, and mild muscle atrophy with limb shortening leading to a mechanical lameness. The other dog had occasional proprioceptive deficits at the 10 week follow-up visit. This resolved completely by six months, however it was not recorded as it occurred after the conclusion of this study.

Radiographic evaluation

Immediate postoperative and follow-up radiographs were available for all dogs. Signs of progressive integration of bone at the site of rhBMP-2 infused CRM and osteotomized bone were noted on all radiographs, with time to healing varying from seven to 20 weeks (median: 10 weeks) (► Figure 1 A-H). The two dogs that had distal humeral condylar fracture nonunions (1 radiation necrosis fracture nonunion and 1 incomplete ossification nonunion after 4 previous surgeries) healed but at a slower rate than diaphyseal fractures (4 and 5 months respectively). There were no noted adverse effects to the rhBMP-2 CRM graft recorded in the cases treated with antibiotic beads intra-operatively.

Discussion

This cohort longitudinal observational study demonstrated successful use of fracture fixation combined with regenerative technique to provide good outcomes in cases with otherwise guarded to poor prognoses. Nine limbs had return to full function and two limbs had acceptable function (28).

The fractures included in this study subjectively had a high risk of failure if revised with the standard-of-care of fixation with autologous or allogeneic bone graft. Four limbs had multiple surgical procedures prior to use of rhBMP-2 on CRM. Three of the four limbs had standard of care fixations for the fractures and some had autologous or allogeneic grafts placed at the time of the original surgery. Due to bone quality, multiple drill holes in the bone, and the avascularity from multiple approaches, these four patients were subjectively considered likely to fail traditional repairs. Seven limbs were treated with CRM and rhBMP-2 after one failure. After osteotomy of the dead bone, many had critical sized defects created. Although the previously implanted bone plates were correctly sized for when bone can share the load, they were likely to be inadequate as bridging implants. The goal of using the CRM and rhBMP-2 after only one failed surgery was to fill the defects and to accelerate bone healing, leading to bone-implant load sharing more rapidly. A previous study showed that rhBMP-2 treated fractures were stronger and stiffer sooner than untreated fractures (29). Clinically, rhBMP-2 treated limbs had increased peak vertical force and vertical impulse, translating to load-bearing sooner, thereby, improving mechanical signalling for further bone formation (29). The other three dogs were high risk due to one already being an amputee, one having radiation necrosis and dead bone that could not be resected, and one being an oligotrophic-atrophic nonunion with a significant sized defect.

Although all cases healed after rhBMP-2 grafts, there was a delay in that response when used in both humeral condylar fractures compared to diaphyseal fractures. One reason for this may be that only a plug type of CRM graft could be placed in the

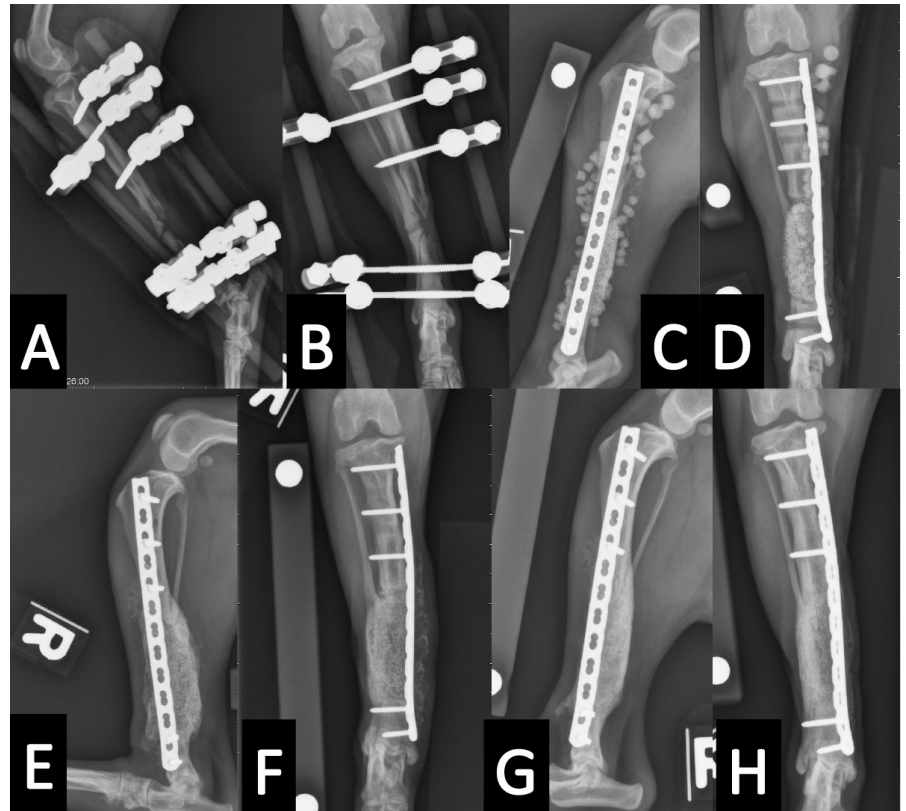


Figure 1 A, B) A one-year-old rat terrier with previously open suspected atrophic nonviable nonunion fracture of the right tibia, previously secured with a type-II external fixator. C, D) Medio-lateral and cranio-caudal radiographs immediately postoperatively, following compression resistant matrix (CRM) with recombinant human bone morphogenetic protein 2 (rhBMP-2) placement along with antibiotic beads^d and fracture fixation with a 2.0 mm locking compression plate. E, F) Medio-lateral and cranio-caudal radiographs four weeks postoperative. Resorption of the antibiotic beads and CRM is evident, along with significant new bone formation and callus. G, H) Medio-lateral and cranio-caudal radiographs obtained 10 weeks postoperatively, demonstrating remodelling with smooth cortical transition between native and new bone.

condyle of one case, limiting the surface area impacted by the graft. Additionally, use of lag-screw fixation in both cases could have crushed the CRM graft leading to changes in the rh-BMP-2 elution from the graft, thus delaying the response. Despite the existence of literature supporting use of rhBMP-2 to reverse the negative effects of radiotherapy in the mandible in rats, the long-term effects of radiation on appendicular bone response to bone morphogenetic protein and the mesenchymal cell population are unknown and could have contributed to the delay in bone healing (30–31).

The value of using antibiotic drugs in the form of antibiotic beads (2 dogs) and orally following surgery (4 dogs), in view of the fact that only one had a positive cul-

ture, is controversial. The presence of bacteria contributing to nonunion fractures is debatable and likely to be under-diagnosed. A study that evaluated molecular diagnostic versus traditional cultures in 24 cases of bone nonunions in humans discovered bacteria in 30 samples via molecular diagnostics, whereas only eight of those samples demonstrated positive culture (32). One retrospective multicentre study found 20% of nonunions treated in humans had positive intra-operative cultures at the time of definitive surgery with the absence of any signs of gross infection or preoperative symptoms. Of these, only 78% healed after the initial procedure, and 92% healed with subsequent procedures (33). Theoretically, avascular bone provides an ideal environment for bacterial growth,

and the effects of such an infection on bone healing are not well-established. When other causes of nonunion such as instability or critical defect cannot be established, infection should be considered.

All dogs returned to full or acceptable function after treatment with CRM and rhBMP-2 despite some complications. Significant inflammation and swelling were most apparent three to five days postoperatively, and were not associated with any further incisional complications or dehiscence. This has been reported as a bone morphogenetic protein-induced inflammation in numerous studies, generally appearing at three to five days and resolving in two to three weeks (18, 34–38). The inflammation is secondary to chemotactic effects of rhBMP-2 on inflammatory cells including mono- and polymorphonuclear cells and osteoclast-like cells (34, 37). Consistent with these studies, the inflammation noted in the reported cases was determined to be physiologically appropriate and not likely to be detrimental to the healing process. Bandaging was used in some dogs because of this anticipated inflammation (18, 34–37). Other dogs had only a semi-occlusal covering over the incision. Bandages can be beneficial as well as detrimental and led to the only two major perioperative complications. We recommend that if bandages are used, they should be removed or changed every few days to monitor this inflammation.

The short-term and long-term complications were not directly related to the treatment of the nonunions with CRM and rhBMP-2. They were related to joint range of motion, minor fracture alignment mistakes and shortening of the limb leading to mechanical lameness. The cause of peripheral proprioceptive deficits after treatment in one dog was never determined. It is possible that removal of the bilateral external fixator caused a common fibular nerve neuropathy.

Compression resistant matrix rather than any other graft carriers was chosen in this study due to the gap size and compressive force on a weight-bearing fracture site, as well as successful reported use in mandibular nonunion fractures (24). Other studies using other carriers have also documented successful treatment of nonunions

(38). Comparing graft carriers is beyond the scope of this study and will need future investigation in fracture nonunion.

The dosage of 0.50 mg/ml rhBMP-2 was chosen due to its successful use in the mandibular reconstruction cases without having excessive bone production at the region (23, 24, 26). A lower dose may still allow adequate bone production in diaphyseal fractures; previous studies showed that a dose of 0.20 mg/ml was successful in 1 mm tibial osteotomy gaps (29). An optimal dose of rhBMP-2 for clinical cases has not been determined, but the dose used in our cases did not cause excessive bone production at the fracture gaps, yet allowed complete union and function. Higher dosage of rhBMP-2 is associated with excessive initial bone formation with resolution within several months and has been reported to promote lower quality bone and instigate a detrimental inflammatory response (34, 39). Our study did not evaluate varied concentrations of rhBMP-2, but did demonstrate success without negative outcomes with the concentration used.

Evidence of bone proliferation was noted on all follow-up radiographs. In previous histological studies, CRM infused with rhBMP-2 yielded well-mineralized trabecular bone, consistent with healthy bone turnover and remodelling (25, 34, 39). Inconsistencies in timing of postoperative radiographs were due to the discretion of the surgeon based on clinical appearance of the patient as well as owner compliance with recommended follow-up. Further studies are warranted to evaluate more thoroughly the rate and histological progression of regenerated bone.

This study was limited by its clinical nature. Case number was limited and encompassed patients with a variety of ages, nonunion fracture classifications and locations, times to repair with rhBMP-2, defect sizes, and repair techniques. Evaluation of carrier types and rhBMP-2 concentration was beyond the scope of this study.

This study demonstrates the return to function of dogs with nonunion fractures that were complicated by infection in some cases, using CRM with rhBMP-2 accompanying fracture fixation.

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Conflict of interest

The authors report no conflicts of interest relating to this report.

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