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Clinical and radiographic evaluations of healing femoral fractures managed with conventional and novel *allo*-cadaveric bone plates in dogs

BS Aliyu[1]*, AZ Hassan[1], GE Ochube[1], BN Umar[2], M Lawal[1] & AS Ibrahim[3]

1. *Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Nigeria*
2. *Department of Veterinary Microbiology, Ahmadu Bello University Zaria, Nigeria*
3. *Department of Theriogenology and Production, Ahmadu Bello University, Zaria, Nigeria*

*Correspondence: Tel.: +2348060279654; E-mail: bashirsaliyugital@gmail.com

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Abstract

Femoral diaphyseal fractures are usually amenable to reduction with the use of orthodox fixation implants, which in most cases are expensive and cause intense stress to the patient, hence the need for safer, new biomaterials. This study assessed the use of *allo*-cadaveric bone plates (CBP-A) and conventional bone plates in managing femoral fractures in dogs. A total of four 8-12 kg Nigerian indigenous dogs were randomly divided into two groups, with each consisting of a male and female dog. Sterilized osteotome wire was employed to surgically create transverse mid-shaft femoral fractures in all the dogs. The fractures in Group I dogs were managed using Vitallium-alloy bone plates and served as control, while Group II fractures were reduced and fixed using CBP-A. Clinical and radiographic assessments for three months were carried out to compare the fracture healing between the groups. Results showed an early stabilization of vital parameters with a premature attempt to use the operated limb on days 4 and 5 post-reduction in groups I and II, respectively. The radiographs revealed good fracture reduction and fixation in all the dogs with the gradual disappearance of the fracture line, as well as progressive bone remodelling as the fracture healing advanced through the sixth week. At 12 weeks, there was distinct medullary and cortical continuity in all the dogs. Therefore, the novel CBP-A used in this study has effectively provided the needed fixation stability with minimal external immobilization for the repair of dog femoral fractures; hence, it should be recommended for use.

Keywords: Biodegradable implants, Cadaveric bone tissue, Conventional bone plates, Cortical screws, Diaphyseal fractures

Introduction

Bone fractures are one of the most common orthopaedic threats in small animal practice. Fractures of the femur represent about half of all long bone fractures in dogs and cats (Unger *et al*., 1990; Gadallah *et al*., 2009). Fracture reduction involves the restoration of normal bone length and alignment and highlights how well a fractured bone has been re-apposed (Gadallah *et al*., 2009; Pozzi *et al*., 2021). The ethic is to manage the fracture in a manner that will restore normal anatomy and maintain the reduction position through the fixation technique (Delaspenas *et al*., 2011). The modalities commonly employed to achieve the desired aim include external coaptation, as well as internal and external skeletal fixations (Endo *et al*., 1998; Minar *et al*., 2013).

Similarly, femoral diaphyseal fractures are primarily managed via internal fixation using vitallium alloy or titanium alloy plates, screws, or intramedullary pins; and external immobilization (Endo *et al*., 1998; Simpson & Lewis, 2003; Piermattei *et al*., 2006; Inas *et al*., 2012). This according to Beale (2004), owe to the fact that most of these fractures are usually the closed type, due to heavy overlying muscles around the thigh area. Factors like surgeon's preference, the configuration of the fracture, the viability of regional soft tissues, presence or absence of microbial contamination, and various clinical factors may influence the choice of implant to be used for the fracture repair (Soontornvipart *et al*., 2003). Moreover, conventional implants are both difficult to come by and quite expensive, posing a challenge of affordability (as regards reduction cost) to most local dog owners in Nigeria (Emmanuel, 2010). Conversely, newer, readily available and cost-effective techniques would not only pave way for smooth fracture management, but might equally overcome the well documented complications of fracture instability, implant migration, failure and or rotation mostly encountered when managing femoral fractures in dogs (Ramesh, 2013).

Bone grafts (autologous and allografts) are another innovative option used to promote bone healing through osteogenesis, osteoconduction and osteoinduction (Albrektsson & Johansson, 2001; Elsalanty & Genecov, 2009). Bone grafting has been in practice for quite a long time, especially when defects or segment losses are involved (Wang & Yeung, 2017). However, in addition to the need for being thermally nonconductive, sterilizable, and readily available at a reasonable cost, an ideal bone graft should be a material that is biologically inert, osteoinductive, osteo-conductive, easily adaptable to the site in terms of shape and size, and replaceable by the host bone (Campana *et al*., 2014; Ferdiansyah *et al*., 2017). Therefore, clinical assessment for efficacy and safety of any biomaterial implants must be conducted before clinical application as earlier recommended by Väänänen (2009). Interestingly, to the best of our knowledge, there hasn't been any report as to the use of processed cadaveric bone tissues as bone plate implants for repair of fractures in dogs. Thus, this study assessed the comparative healing of canine femoral fractures by evaluating clinical and radiographic features of healing when managed with a*llo-c*adaveric bone plates (CBP-A).

Materials and Methods

Experimental dogs

A total of four Nigerian indigenous breed of dogs weighing about 8-12kg (consisting of both sexes) were used for this study. Ethical approval for the use of dogs in this study was granted by Ahmadu Bello University Committee for Animal Use and Care, with approval number: ABUCAUC/2019/26. The dogs were acquired from local dog owners, and housed within the kennels of the Small Animal Clinic Unit (SACU), Veterinary Teaching Hospital, ABU Zaria. The dogs were fed restaurant leftover, cooked beans, meat and crayfish three times daily, while water was provided *ad-libitum* throughout the conditioning and experimental periods. An acclimatization period of 10-14 days was observed for each dog before the commencement of the study.

Allo-Cadaveric Bone Plates (CBP-A)

Fresh femoral and humeral bones from a dog that died of road traffic accident were sourced from Necropsy Unit of the Veterinary Pathology Department, ABU Zaria. The collected bones were then stripped of muscular and soft tissue attachments using blade, leaving behind the free long bones, which were then air dried. Using hard saw, the proximal and distal epiphyses, and metaphyses were detached, thus transforming the bones into slender fragments of different sizes. The prepared bone fragments were immersed in 35% hydrogen peroxide solution for 30 minutes, then meticulously washed with same solution. Thereafter, they were rinsed with 10% methanol then allowed to air dry; a modification of allograft tissue processing technique (MTF, 2009). The tensile strength of the bone fragments was evaluated, then autoclaved (at 15 ounce per inch, and 161^0C) before usage.

Dimensions of Bone Plates and Screws Used

Standard, straight vitallium-alloy orthopaedic plates of about 75mm length each containing four holes (of 3.5mm diameter), and processed *allo*-cadaveric plates of about 60mm length containing four holes (of 2.7mm diameter) were used in the study. Vitallium alloy cortical orthopaedic screws measuring 3.5mm by 20mm and 3.0mm by 20mm were respectively used to secure the plates onto the fractured bone cortices.

Study design

The experimental dogs were randomly divided into two groups (I & II) with each consisting of a male and female dog. Sterilized osteotome wire was employed to surgically create a transverse mid-shaft femoral fracture in all the dogs. The fractures in Group I dogs were fixed using conventional vitallium-alloy bone plates and served as control, while Group II dogs were managed using processed CBP-A with standard orthopaedic screws. Clinical and radiographic assessments were carried out to compare the progress of fracture healing between the two groups. Immediate post-reduction and follow up radiographs taken at two weeks interval were observed for a period of 12 weeks (healing time).

Pre-operative preparations

All experimental dogs were given antiseptic bath using antiseptic soap, and mild chlorhexidine gluconate solution (0.3%) a day prior to surgical procedure. This was done after proper preparation of the surgical site by liberally clipping the left hind limb from the level of os-coxae to the stifle joint. The surgical site was appropriately scrubbed in accordance with the method of Piermattei and Greeley (Johnson, 2014). Sterile, standard general surgical and orthopaedic packs were utilized for all the procedures. Likewise, aseptic conditions and measures were adhered to throughout the study; and surgical team preparation was performed in accordance with standard procedures (Bojrab, 1985; Knecht *et al*., 1987).

Anesthetic protocol

All surgical procedures were carried out under general anesthesia. The cephalic vein was cannulated; pre-anesthetic medication: Atropine Sulphate (0.02mg/kg) (Amopin®, Yanzhou Xierkangtai Pharma Ltd, China) and Chlorpromazine Hydrochloride (4mg/Kg) (Pauco Pharmaceutical Ltd, Nigeria) were administered through the established intravenous line. Induction of anaesthesia was achieved using Thiopental Sodium (10mg/Kg), while Ketamine Hydrochloride (22 mg/Kg) (Pauco Ketamine Injection®, Kwality Pharmaceutical Ltd, India) was employed for maintenance of anesthesia.

Surgical approach to femoral shaft

Lateral approach was employed in accessing the femoral shaft. The dog was placed on right lateral recumbency with the prepared left thigh area uppermost on the surgical table. Using a scalpel blade, skin incision was made over the cranial border of the bone from the sub-trochanteric area to a point near the femoral condyles. Subcutaneous fat and superficial fascia were incised directly beneath the skin as described by Piermattei & Greeley (Johnson, 2014). The incised skin margins were bluntly separated, undermined and retracted, while the *fascia lata* was incised along the cranial border exposing the muscle division between the *biceps femoris* caudally and the *vastus lateralis* cranially. On locating the point of division of the two muscles, fascial incision was extended with scissors and the biceps muscle was retracted caudally to expose the femoral shaft. The adductor muscle which is firmly attached to the caudal border of the femoral shaft, was bluntly reflected subperiosteally freeing the bone at this point. In the same manner, the *vastus-intermedius* muscle on the cranial surface of the shaft was retracted by freeing the loose fascia between the muscle and the bone (Plate I).

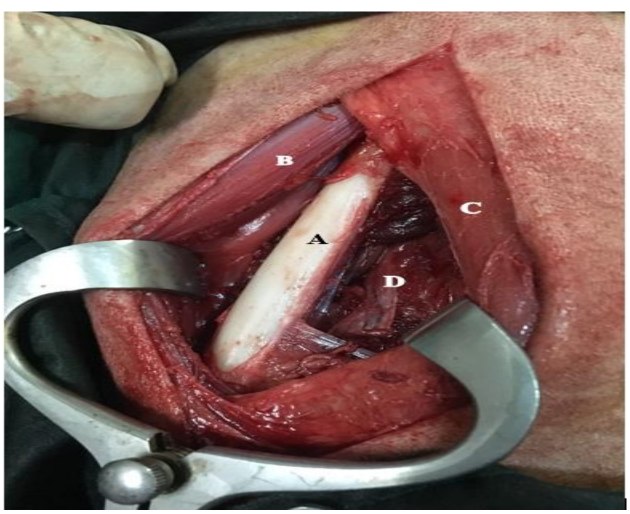

Plate I: Exposed femoral shaft (A), *vastus lateralis* (B) and *biceps femoris* (C) muscles. Note the point of insertion of the *adductor magnus* (D) muscle being freed from the caudal border just beneath the biceps

Creation of the experimental fractures

Using a sterilized osteotome wire wrapped around the mid femoral diaphysis, the two ends (handle of the wire) were continuously pulled in an up/down manner by both hands until a complete bony discontinuity was created at the mid-shaft of the femur. While this was going on, normal saline solution was sprinkled intermittently unto the site to prevent the bone tissues from dying out due to frictional pressure and heat of the osteotome wire. The fracture segments (proximal and distal) were exposed before alignment and reduction (Plate II). Fracture reduction: After establishing a complete mid-diaphyseal femoral fracture, proximal and distal fracture segments were manually held (angled) upwards and gently pushed downwards (angulation and tugging), and the aligned fractured bone was held with a bone clamp before fixation. Appropriate vitallium and *allo-cadaveric* bone plate sizes

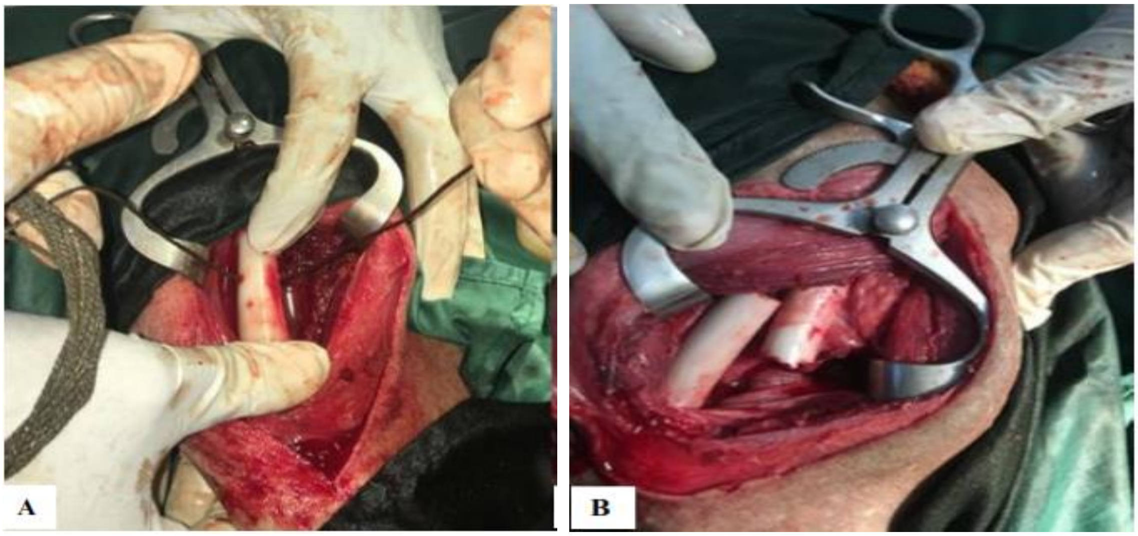

Plate II: Creation of diaphyseal femoral fracture using osteotome wire (A), and the two fracture segments overriding each other (B)

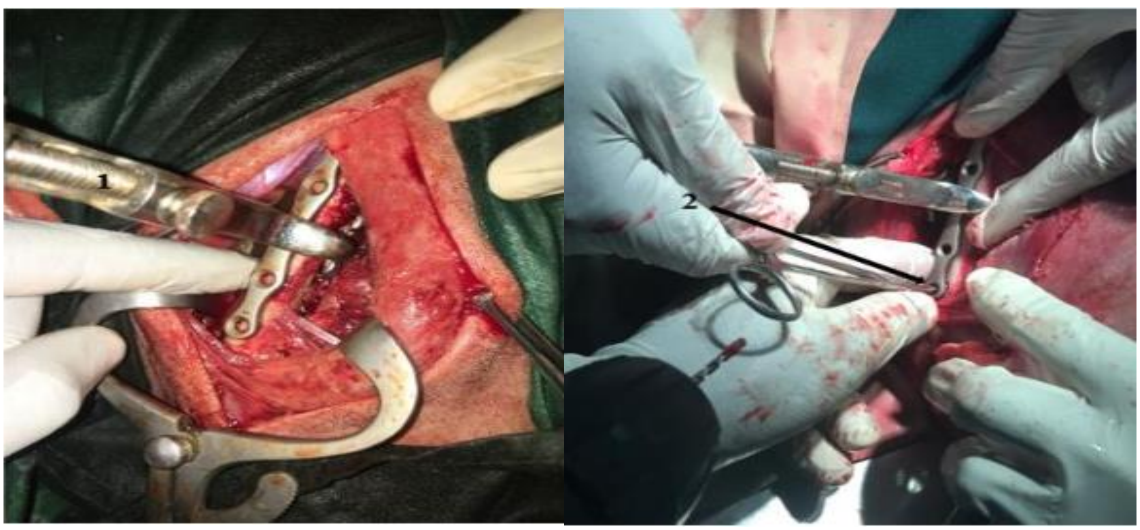

Plate III: Anchoring Vitallium alloy plate unto bone surface with bone clamp (1) and drilling of screw holes on the cortices (2)

corresponding to the dog's femoral length were then selected and positioned on the lateral surface of the femoral diaphysis (proximal and distal segment, at equidistance between them), which is the tension side of the fracture. Using bone holding clamps, each plate was firmly anchored directly to the lateral diaphyseal surface along its length and midway each on proximal and distal fracture segments sharing equal number of screw holes (Plate III & IV). Holes were drilled penetrating 4-6 cortices (2-3 on proximal and distal fracture segments) using an automated electric drill at low speed (< 150 RPM) with an attached 3.0mm diameter drill bit, corresponding to 3.5mm cortical (threaded) screws. All intended screws were manually driven using a tension screwdriver.

Surgical site Closure: Following the achievement of the fracture reduction and fixation, closure of the surgical site consisted of suturing the *fascia lata* to the cranial border of the biceps muscle by a simple continuous pattern of absorbable suture (size 1 chromic catgut, Helm® Germany). For wider approach, the origin of the *vastus lateralis* muscle was also sutured to the gluteal tendon insertions on the greater trochanter and the superficial gluteal is re-attached to its tendon with interlocking mattress pattern using absorbable chromic catgut size 2. Skin incision was closed using monofilament Nylon suture

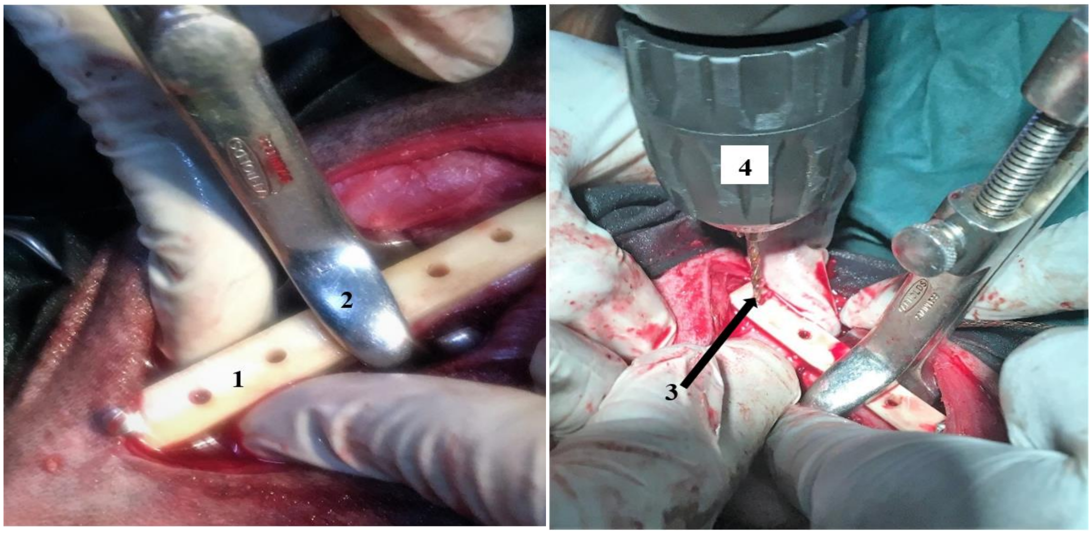

Plate IV: Anchoring of *Allo*-cadaveric bone plate (1) onto a fractured bone surface using bone clamp (2); and drilling of the screw holes on the cortices (3, 4) before subsequent plating of the fractured bone

size 1 (3 metric) in a horizontal mattress pattern. 10% povidone iodine solution (WOSAN®) was applied along the suture line after mild cleaning with chlorhexidine solution. Two layers of sterile gauze and adhesive plaster were used in dressing the surgical wound. Consequently, a modified Robert-Jones bandage (using cotton wool + gauze pads and crepe bandage) was then applied on the operated limb to minimise postoperative swelling, while Robinson sling was applied to prevent weight bearing on the limb.

Post-operative Care

Following recovery from anesthesia, improvised Elizabethan collar was applied on each dog. An analgesic, Diclofenac sodium injection (4mg/Kg) (Diclowin®, Chupet Pharm. Ltd, China) was given for 3 days, intramuscularly. Procaine penicillin injection (20,000IU/kg) (Gossipain® Shanxi Federal Pharmaceutical Ltd., China) and Streptomycin (20mg/Kg) (Paulio® Shandong Reyoung Pharmaceutical Ltd., China), were given for 5 days, intramuscularly. Skin sutures were removed at day 14.

Post-operative clinical and radiographic examinations: Physiological parameters; rectal temperature, respiratory rate, and pulse rate were monitored and recorded daily throughout the recovery period. Clinical assessment was done for both groups by subjecting the dogs to ambulation test weekly starting from the post-anesthetic recovery time. The time of first weight bearing on the affected limb was noted. Lameness was assessed weekly and was graded as present or absent. Radiographic exposures of immediate post-operative radiographs were taken using MDX-100 F/S Medical X-ray machine (Suzhou, Jiangsu, China) with exposure factors of 70kVp, at 50mA; Medio-Lateral and Cranio-Caudal views and were repeated at two weeks interval, four weeks until complete union was achieved after 12 weeks (Hassan & Hassan, 2003; Easton, 2006). The radiographs were evaluated to estimate angulation, maintenance of fracture reduction and rate of bone healing. For bone healing assessment, the radiographic results were graded as excellent, good, fair, and poor, as described by Gian *et al*. (2009). For the CBP-A group II, osseointegration and dissolution of the implant were also qualitatively evaluated.

Results

The creation and reduction/fixation of the femoral fractures were successful in all the dogs. Reduced and fixed fractures were stable and immobilization was effectively achieved for all the dogs. Post-operative surgical wound healing was good for all the dogs.

The mean post-surgical vital parameters for both groups were slightly above the mean pre-surgical values. This rise reached its peak value at day 2 post-operatively, and was least observed in Group I. By post-operative day 7, all values were relatively within normal ranges for all the dogs except for a dog in Group II. Immediately after recovery from anaesthesia, non-weight bearing lameness was observed for all the dogs as they all adducted the operative limb. Four days post-operatively, attempt to bear weight on the operated limbs was first observed in Group I and by the 5th post-operative day for Group II. The attached Robinson's sling was removed at 8 and 12 post-operative days for all the dogs in Group I and II respectively after noticing partial weight bearing on the operated limb, but with some degree of lameness. Skin sutures were removed at two weeks post-operatively in both groups. At four weeks post-reduction, Group I and II dogs comfortably bore weight on the operated limb with slight lameness and by 5th -6th week post-operative, the dogs could run on the limb. However, in spite of the full weight bearing on the operated limb by all the dogs, slight degree of lameness was observed even at week 12 post-reduction.

In both groups, immediate post-operative radiographs showed good alignment and fixation of the fracture with clear fracture/inter-fragmentary lines, good apposition and plating stability on the radiographs (Plate V). At two weeks post-reduction, radiographic exposures revealed the presence of osteogenic activity, minimal soft tissue and periosteal reaction, with maintained alignment in both groups. Likewise, minimal but visible callus formation with gradual obliteration of the fracture line was seen in *allo*-cadaveric group (Plate VI). There was progressive callus formation obliterating the entire fracture line at four weeks post-reduction in both groups of dogs (Plate VII). Also, the CBP-A implant-cortical bone

Plate V: Immediate post-operative Medio-lateral and Cranio-caudal radiographs of transverse femoral diaphyseal fracture managed with vitallium alloy bone plate (A & B), and *allo*-cadaveric bone plate (C & D). Good plating stability and alignment with obvious fracture gaps (white arrows) are seen

Plate VI: Medio-lateral and Cranio-caudal radiographs of transverse femoral diaphyseal fracture reduced and fixed with vitallium alloy bone plate (A & B), and *allo*-cadaveric bone plate (C & D) at two weeks post-reduction. Group I showed good apposition but minimal periosteal reaction with incomplete obliteration of fracture line (white arrows); while Group II revealed high osteogenic activity, soft tissue and periosteal reaction with very thin fracture gap (white arrows) interface was significantly reduced at six weeks post-reduction making it difficult to locate the fracture line on the radiograph. Thus, all the dogs had a good and progressive remodelling with complete obliteration of the fracture line. Good fracture healing with evidence of advanced bone remodelling, cortical bone thinning, and increased medullary space were observed at eight weeks post-reduction in all the fractured dogs. Similarly, gradual decrease in density of the CBP-A was noticed (Plate VIII). Consequently, cortical and medullary continuity, complete fractured bone remodelling with the apparent restoration of the normal bone anatomy, was observed in all the dogs at 12 weeks post-operative (Plate IX).

Discussion

This study was specifically centred on fabrication of bone fixation implants from dog cadaveric bone tissues and subsequent determination of the implants' *In vivo* pre-clinical performance and safety in experimentally fractured dogs. Broadly as documented by Jones (2016), two radiographic views

Plate VII: Medio-lateral and Cranio-caudal radiographs of transverse femoral diaphyseal fracture managed with vitallium alloy bone plate (A & B), and *allo*-cadaveric bone plate (C & D) at four weeks post-reduction. Progressive bone remodelling with almost complete obliteration of the fracture lines, visible periosteal callus (white arrows) & proximal part of the implant had almost fused with the endogenous bone (black arrow, D)

Plate VIII: Medio-lateral and Cranio-caudal radiographs of transverse femoral diaphyseal fracture managed with vitallium alloy bone plate (A & B), and *allo*-cadaveric bone plate (C & D) at eight weeks post-reduction. Advanced bone remodelling (M), cortical and medullary continuity, as well as restoration of bone anatomy, are evident

Plate IX: Medio-lateral and Cranio-caudal radiographs of transverse femoral diaphyseal fracture reduced and fixed with a vitallium alloy bone plate (A & B) *allo*-cadaveric bone plate (C & D) at 12 weeks post-reduction. Healed fracture with complete bone remodelling and restored normal bone anatomy are seen

are needed (and were used in the current study) to avoid the concealment of the fracture lines on the radiographic films. This will also help to ensure proper visualization and interpretation of the fracture healing. Early attempts to use the operated limbs were observed in both groups of dogs by the first week of fracture management with subsequent usage at about four weeks post-reduction, *allo*-cadaveric bone implants may be employed to achieve excellent fracture reduction in dogs. Moreover, as reported by Perren (2002) and Piermattei *et al.* (2006), the goal of any fracture management is to have early ambulation while maintaining minimal surgical trauma and flexible fixation with a stable (rigid) fracture segments following clinical intervention. For the fact that the novel *allo*-cadaveric bone implants used in this study were relatively more robust, much tissue had to be removed before plating, and this could be the reason why the *allo*-cadaveric bone implant group showed little delay (day 5 as opposed to 4th-day post-reduction in control group) in their return to ambulation.

The early rigid fixation achieved in this study as evident by visible callus formation around the fracture line observed on the radiographs of both groups of dogs about two weeks post-reduction; as well as the small inter-fragmentary gap seen might have accounted for the observed primary healing (gap-healing) recorded in the fractured dogs. This statement agrees well with the findings of Yoon *et al*. (2018), who reported that inter-fragmentary gap of about 1mm, absence of micromotion at the fracture site, and rigid fixation favored a more accelerated healing rate with little external callus formation in canine radial fracture management when using locking plates, cortical screws, and mesenchymal stromal cell sheets. Similarly, the observed gradual decrease in *allo*-cadaveric bone-implant density as revealed by increasing radiolucency on the radiographic films from eight weeks onwards might be due to early graft incorporation sequel to a supposed osteointegration and osteoclastic activities which have been documented to initially resorb the implant (being a cortical bone graft) thereby freeing some calcium ions before osteoblasts could initiate osteogenesis (Oryan *et al*., 2014). Interestingly, in both groups of dogs, stable reduction of the fracture and good apposition up to the clinical union and beyond were recorded. This concurs with the standards reported by Piermattei *et al*. (2006), who made it clear that, a good fixation implant must remain in place until the clinical union is achieved.

Moreover bone plates have been reported to be ideally suited for stable fractures of the femur in dogs especially if rigidly secured on stabilized fracture segments, as commonly seen when prolonged healing is anticipated (AbdElRaouf *et al*., 2017). While it is worth mentioning that tissue implant technology entails careful selection of any material intended for use as raw material to produce bone fixation implants, since incorrect use of implants themselves can lead to bone fractures, defects, or impaired (and sometimes delayed) bone healing (Kim *et al*., 2020). Similarly, Meinig *et al*. (1996) and Ito *et al*. (2001)

documented that the mechanical strength of any fracture fixative relative to the fractured bone's flexibility must be ascertained before use, hence, the choice for the cortical aspect of the cadaveric bone tissue in the current research. The success of which should lead to clinical trials.

The *In vivo* pre-clinical trial through clinical and radiographic assessments of the novel *allo*-cadaveric bone-implant tested in the present study yielded good results, which suggest relative safety and makes it a good candidate for clinical trials. The implant was able to support the weight of the experimental dogs (with transient minimal external immobilization) and gives the needed fixation stability at fracture site until healing without any noticeable exaggerated short-term responses. As not much obvious clinical or radiographic differences existed between the vitallium alloy and CBP-A in managing transverse femoral diaphyseal fracture in dogs; the availability, affordability, biodegradability and minimal stress of a secondary procedure for implant removal post-reduction even make the CBP-A an excellent option to use. Therefore, a more robust biocompatibility study of the CBP-A implants to cover their medium and long-term effects, hence, suitability for fracture reductions in dogs is recommended. The study should be extended to field/clinical fracture cases to fully determine the implant's ability in reducing other fractures amenable by plating. Further studies using biodegradable fastening material for the plates (other than Vitallium alloy screws) e.g., screws made from cadaveric bone, to completely eliminate the need for second surgery.

Despite this outstanding performance and problem-solving potentials of our novel cadaveric bone plate implant in this study, some few limitations like implant failure/fracture; shortness of the post reduction monitoring and evaluation period as well as less sensitive immunological test for the implant biocompatility and or rejection (both of which were due to financial constraint/lack of sponsorship); and the smaller sample size for the groups, were encountered.

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Conflict of interest

The authors declare that there is no conflict of interest.

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