



Research article

Humeral fracture treatment in pigeons by bone pins made from ovine and canine bones



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ABSTRACT

The tubular, pneumatic long bones of the bird present unique challenges in veterinary orthopedics. Various traditional and innovative forms of fracture fixation have been applied in bird orthopedic. The purpose of this study was to compare the applicability of the stainless steel intra-medullary pin with the pins made from ovine and canine long bones. Ovine and canine long bones were used to prepare pins in appropriate size and dimension. The pins were treated chemically, hydrated, dried, packaged and sterilized by ethylene oxide. Forty domestic male pigeons were divided into four equal groups. Pigeons were anesthetized by combination of xylazine and ketamine. Humeral bones were cut by a diamond disc burr. In the first group the fractured bones were not treated and was regarded as control group. In the second group stainless steel pins were inserted into the humeral medulla of pigeons; and in the third and fourth groups, prepared pins from ovine and canine long bones were inserted into the medulla, respectively. The operated wing was bandaged to the body and stabilized. Post-operative care included: clinical assessment of surgical wound, wing holding and flight assessment. The flight behavioral assessment and radiographic studies were carried out every two weeks for 32 weeks.

After 32 weeks the pigeons of the first group were not able to fly, in the second group pigeons had imbalance in flight; and in the third and fourth groups the pigeons were able to fly with no problem. Radiographic study showed no significant differences between groups 2, 3 and 4 but there was a significant difference between group 1 and other three groups. None of the bone pins were rejected; all were absorbed in later stages in large quantities.

The IM pins made from long bones of sheep and dog pins can be considered as an appropriate and alternative internal fixation technique, because they are very firm, strong, provide very good internal fixation for bone alignment, showing no tissue sensitivity, no rejection and therefore not necessity to be removed. While the stainless steel IM pin creates imbalance in flight and they have to be removed after bone union is completed, which needs another surgical intervention and stress.

1. Introduction

Despite the fact that veterinary science has made a lot of progress in the field of surgery, there has been little attention paid to orthopedics and repair of fractures in birds. The most common damage to the bones of the birds is fracture. Bone grafting is a technique that has the potential to improve the quality and accelerate the healing of fractures. Bone grafting has very beneficial effects on fracture repair. The use of bone grafts in recent decades has been an integral part of orthopedic surgery in veterinary medicine. Bone grafting is used in cases of non-union, delayed-

union and arthrodesis of joints in various livestock [1]. There are many disadvantages of metal internal fixation in different animals, including osteomyelitis, sensitivities, tumor formation, loosening and detachment of the plate. In addition the birds have small bones and sometimes pneumatic bones, poor fit of the pin with bone and thinness of the bone marrow that does not have the ability to hold the bolt when using the plate [2]. There are also technical problems for placement and removal of the plate such as the lack of appropriate plate sizes, increased exposure to the surgical position and consequently, increased surgical time, prolonged anesthesia and cost [2]. The use of synthetic materials in birds has

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its own limitations such as susceptibility to fatigue and fracture over time and biologically does not combine with the host skeleton. Since most of the fractures in the birds are in the bone of the wings and these bones has a large amount of movement during flight time. Using bone graft for internal stabilization purpose reduces osteoarthritis, accelerates the expansion of bone cells at the fracture site, accelerates the differentiation of mesenchymal cells and other cellular resources into osteoblasts, establishes a solid scaffold for the formation of new bone and provides the necessary minerals for fracture repair [3]. Fractures of the wings induce serious complication in birds [4, 5], which can prevent the bird from flying. For each individual fracture, it takes a certain time for bone repair; this duration depends on the vertebrate species, anatomy of the involved bone, fractured site and degree of bone loss, time lapsed, extend of soft tissue damage and host factors including age, individual variations and systemic conditions [6, 7]. The main structure of the bone is hydroxyapatite crystal network. The maximum stress on the bone is at its two ends. The outer shell of the bone has effective surface for resistance to various forces. The humerus is a pneumatic bone in the birds, therefore the cortical bone is fragile and needs special attention during surgery otherwise it could break apart into pieces easily [8, 9].

To have an effective bone healing, the bone needs to have a sound stability and a reasonable blood supply of oxygen, healing cells, growth factors, and micro-nutrients, therefore if a fractured bone is left unstable or lacks blood supply, it can lead to nonunion [10]. As a biologic process, fracture healing has a complex specific regeneration and gene expression pattern, and can be performed by primary (direct intra-membranous bone formation) and secondary (endochondral bone formation) bone healing. Large defects usually heal by secondary pattern due to micro-motions; but in simple fractures with minimal defect, the primary pattern occurs [11] if excellent stability provided. Previously, poly-dexonin pins were used in the pigeon arm bone instead of metal pins. This type of pin has a biological absorption and does not require surgical removal of the pin [12]. However, the effects of chemical polymers on the patient's body have not yet been reported. The external fixation techniques has been used for bird fracture repair [13, 14]. Polymethyl methacrylate (PMMA) was used inside the bone cavity, which required 10 min time to be cured and cemented the two fracture fragments as an internal stabilization [15]. Slow hydrolyzing pins, such as poly lactic acid, have been used in different animals.

In this study we prepared bone pins from the long bones of the dog and sheep to use them in the pigeon humeral fracture which is a pneumatic bone, since there are many referral cases of different species of the birds referring for fracture repair. This study was evaluated by clinical and radiographic examination, comparing the repair process, repair time, absorption of the bone pins, and ability to fly.

2. Materials and methods

Forty pieces of native and domesticated male, young adult pigeons (weighing 500 ± 60 g) were prepared from a certified supplier and used in this study. The study was approved by the Research Ethics Committee of Shiraz University (13790214); and the steps were complied with ARRIVE guidelines [16]. They were housed and fed in a room (22°C , with day light), which were free to move. The long bones (tibia and femur) of sheep were collected from the slaughter house. The long bones (tibia and femur) of an euthanized dog due to non-infectious incurable situations were prepared. The soft tissues of the bone were removed, they were cut into pieces (5–7 cm long, 4 mm width), and they were sand by Sand machine to shape the bones similar to pins in a length and diameter adjustable to the marrow size of the humeral bone (Fig. 1). Then they were treated chemically by hydrogen peroxide to degrease the bones, hydrated, dried in room temperature, packaged and sterilized by ethylene oxide. Then pigeons were randomly divided into four groups of ten. Birds were off feed for 2 h prior to surgery, they were anesthetized by IM injection of mixture of 1 mg/kg xylazine (Xyla, Interchemie, Holland) and 25 mg/kg ketamine (Alfasan, Woerden Holland). Then they were restrained on the table on dorsal position and medial side of humeral bone was prepared for aseptic operation. Following skin incision and muscle splitting the categorizations of different treatments were done randomly (using Random Number Table) as below. Each group consist of ten pigeons according to statisticians consult, considering the animal rights and costs.

Control Group: The mid shaft of the humeral bone was cut by a diamond disc burr inducing a transverse fracture. The surgical site was sutured normally. The wing was sprayed by 10% betadine solution and bandaged to the body. This group was considered as negative control group.

Conventional Metal Pin (CMP) Group: The mid shaft of the humeral

Table 1

The status of operated wing holding.

Group	Week					
	2	4	6	10	20	32
Control	50% WH	10% WH	Normal	Normal	Normal	Normal
CMP	20% WH	10% WH	Normal	Normal	Normal	Normal
OBP	Normal	Normal	Normal	Normal	Normal	Normal
CBP	20% WH	Normal	Normal	Normal	Normal	Normal

The table shows the status of operated wing holding normally (Normal) and wing hanging (WH) post operation till 32 weeks. Control group: the fracture was not fixed; CMP group: the fracture was fixed by conventional metal pins; OBP group: the fracture was fixed by ovine bone pin; CBP group: the fracture was fixed by canine bone pin.

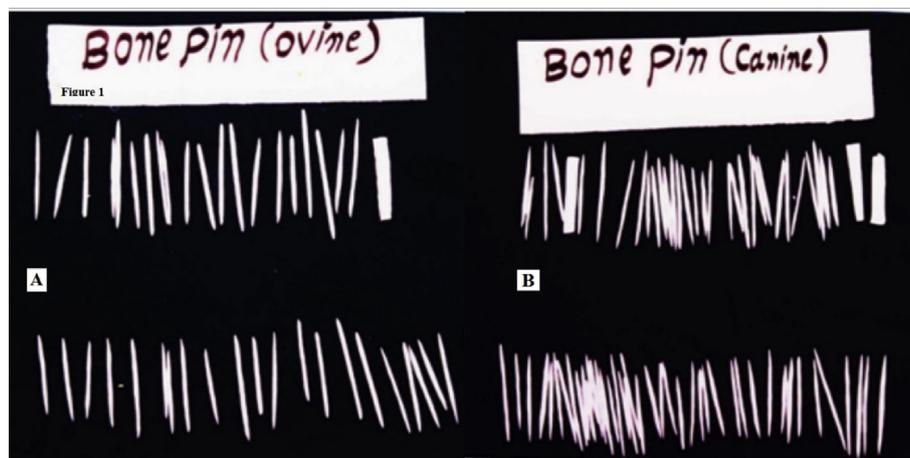


Fig. 1. Bone pins. A. Pins made of sheep's long bones (Tibia and Femur) and B. pins made of dog's long bones (Tibia and Femur).

Table 2
The status of winging and flight.

Group	Week					
	2	4	6	10	20	32
Control	NW	NW	NF	NF	IF 100%	IF100%
CMP	NW	W 80%	IF 100%	UF 90%,	UF 90%	UF 90%
				NF 10%	IF 10%	IF 10%
OBP	NW	W 80%	IF 100%	CF 90%	CF 100%	CF 100%
CBP	NW	W 70%	IF 100%	CF 80%	CF 100%	CF 100%

The table shows the status of winging and flight post operation till 32 weeks (NW: no winging, NF: no flight, W: winging, CF: complete flight, IF: incomplete flight, UF: uncontrollable flight). Control group: the fracture was not fixed; CMP group: the fracture was fixed by conventional metal pins; OBP group: the fracture was fixed by ovine bone pin; CBP group: the fracture was fixed by canine bone pin.

bone was cut by a diamond disc burr inducing a transverse fracture and conventional metal pin (2 mm in diameter, 3 cm length and average weight of 1.035 ± 0.068 g) was used to fix it. The surgical site was sutured normally. The wing was sprayed by 10% betadine solution and bandaged to the body. This group was considered as positive control group.

Ovine Bone Pin (OBP) Group: The mid shaft of the humeral bone was cut by a diamond disc burr inducing a transverse fracture and was fixed by pin made from sheep bones (2 mm in diameter, 3 cm length and average weight of 0.513 ± 0.014 g). The surgical site was sutured normally. The wing was sprayed by 10% betadine solution and bandaged to the body.

Canine Bone Pin (CBP) Group: The mid shaft of the humeral bone was cut by a diamond disc burr inducing a transverse fracture and was fixed

by pin made from dog bones (2 mm in diameter, 3 cm length and average weight of 0.472 ± 0.012 g). The surgical site was sutured normally. The wing was sprayed by 10% betadine solution and bandaged to the body. This dressing was not changed till the 2nd week, in order to avoid any motion to the fractured site.

2.1. Postoperative care

The pigeons were treated by Enrofloxacin 10 mg/kg (SC) for three days (Enrofloxacin, Baytril Bayer, Germany). Post-op analgesia was provided by Aspirin 20% syrup (Kirkland, Vita health product, Canada) in drinking water for five days. They were evaluated clinically for problems of the surgical site, wing holding position, and the status of winging and flight every two weeks till the 32nd week post operation. Radiographs were prepared from the operated wing post operation and on weeks 1, 4, 6, 8, 10, 12, 14, 20, 26 and 32 (MS16, 20 MAS, 45KV). Not all the pigeons were called on 32 post-op week for further evaluations. The radiographs were evaluated for the status of the pins (over ride, displacement, alignment, resorbtion and pin fracture), internal and external calluses, and repair status (delayed union, non-union, and remodeling) by scoring as 0 (not good), 1 (mild), 2 (good) and 3 (very good) statuses. The radiographs were read by two radiologists separately in a blind manner. Non-parametric repeated measured analysis (and Mann-whitney test as post hoc analysis) was done for the mean score of each group during the time of study; and Kruskal Wallis analysis (and post hoc Mann-whitney test) was performed for statistician analysis between different groups in each week of study. The analyses were performed using an SPSS package (SPSS 24 for Windows, SPSS Inc, Chicago, IL, USA). P-values ($P < 0.05$) were considered statistically significant.

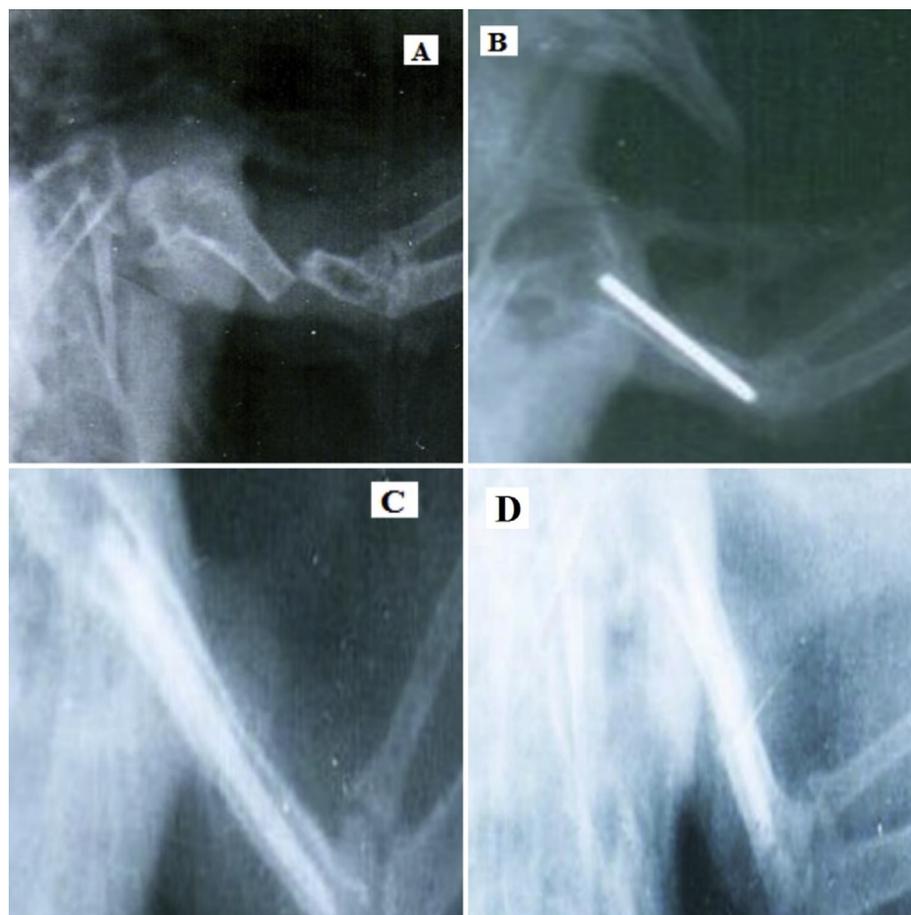


Fig. 2. Fracture of pigeons humeral bones of different groups at 14 post-op week. A. The control group with no fixation; B. the CMP group with conventional metal pin fixation; C. the OBP group fixed by pin made of ovine long bone; and D. the CBP group fixed by pin made from canine long bone.



Fig. 3. The handmade bone pins made from sheep's bones showed to be more dense and solid than the pins made from dog's bones. A. Ovine pin. B. Conventional Metal Pin. C. Canine Pin.

3. Results

Clinically, in all groups there were some degrees of edema and inflammation in the surgical site which was improved by the second week. In terms of holding position of the wings the results were

summarized in Table 1. Also the results of the ability of pigeons to fly completely, incomplete fly and uncontrollable flight was presented in Table 2.

In the control group, from the fourth week, some periosteal callus was formed, until the fourth week most pigeons in this group exhibited

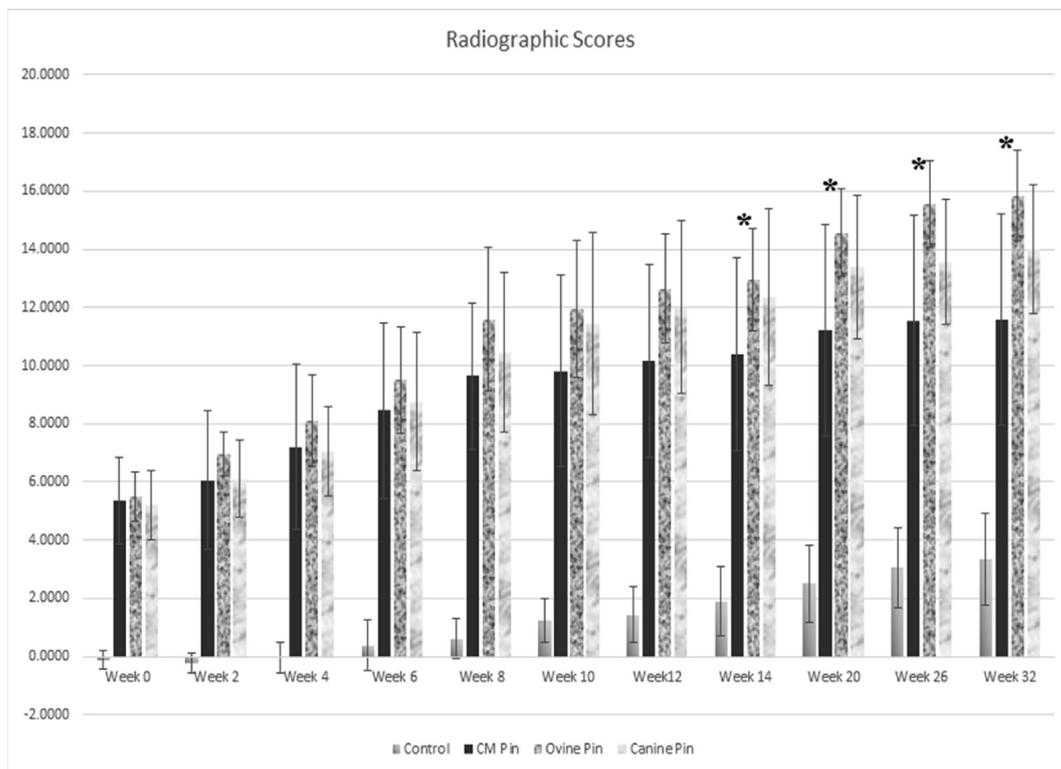


Fig. 4. The histogram shows the mean ± SD of scores given to different radiographic findings of humeral bone repair in the four groups during the 32 weeks of study. The changes over time in each group were significant between different weeks ($P < 0.05$). The control group had statistically significant differences with other groups ($P < 0.01$). (*) shows the 14th week that the metal pin and ovine bone pin groups started to differ significantly with each other ($P < 0.05$) that continued till the 32nd week. But other pin groups showed no significant differences with each other in no weeks.

negative radiologic scores (Fig. 2 A). The metal pins were completely stable in the humeral bone in all members of the second group throughout the study only creating imbalance in winging and flight (Fig. 2 B). In the third group, the bone pins made from the sheep bone retained the fracture fragments well in place, indicating solid union; here, the bone pins showed resorption and remodeling of the intra-medullary canal as well as cortical surface that was optimal compared to normal conditions (Fig. 2 C). In the fourth group, the bone pins made from canine long bone retained two fractures fragments well enough indicating solid union similar to the third group; the bone pins showed resorption and remodeling similar to the third group (Fig. 2 D). However, the bone pins made from ovine bones showed to be more dense and solid than the pins made from canine bones (Fig. 3).

Radiographic scores of each group showed a significant increase from the first week (week zero, immediate post-op radiograph) to 32nd week; Kruskal Wallis analysis showed that the differences were significant between control group and the other three groups ($P < 0.001$) (Fig. 4). From the 14th week, the metal pin and ovine bone pin groups also showed significant differences with each other ($P < 0.05$) that continued till the 32nd week. But metal pin and canine bone pin groups, and also canine and ovine bone pin groups showed no significant differences with each other in no weeks. Non-parametric repeated measured analysis also showed significant changes between different weeks of study in all groups (Fig. 4). In the control group, at 6th week radiographic scores differed significantly with other previous weeks till the 14th week, after that the results differed non-significantly with the next other weeks. In the metal pin group, at 4th week radiographic scores differed significantly with other previous weeks till the 8th week and after that the results differed non-significantly with the next other weeks. In the ovine bone pin group, at 2nd week radiographic scores differed significantly with the previous week (week 0) and continued till the 20th week, after that the results differed non-significantly with the next other weeks. In the canine bone pin group, at 4th week radiographic scores differed significantly with other previous weeks till the 12th week, and afterward the results differed non-significantly with the next other weeks.

The study of radiographs showed that bone density of the bone pins made from sheep's and dog's long bones declined over time, with bone pins largely absorbed by the 32nd post-operative week. Fig. 5 revealed that in the control group with no fixation, the healed humeral fracture is shorter than the intact one of the same pigeon; compared to the healed humeral fractures fixed by metal conventional pin and pins made of ovine and canine long bones.

4. Discussion

Flight is an important activity in birds, and any disruption or inability to fly endangers the bird's life. PLLA (Poly-L-Lactic acid) isomer has been used for thigh bone fracture repair in rabbits and in femoral fracture in cats and dogs [17]. The PLLA is hydrolyzed in the first four to five months and converted to lactic acid [14]. Therefore, the use of substances that gradually absorbs in the body is a new orthopedic area; and this research was carried out to compare pins made of dog's and sheep's long bones and metal conventional pins in the experimentally induced humeral pneumatic bone fracture in pigeons. The total analysis period was extended to 32 weeks to observe the remodeling status of the healed bones.

Providing a rigid fixation is the first important step in orthopedic procedures and fracture healing. This factor could not be achieved in the control group with no implant fixation. Therefore the radiographic scoring data of the control group had significant differences with other three groups till the 32nd week. Also micro motions caused by no rigid fixation [11, 18] leads to shortening of the healed long bone, which is observed in the control group of this study. Other three groups demonstrated similar healing pattern till the 14th week that conventional metal pin and ovine bone pin started to differ significantly with each other. This significant difference continued until the last week of the study. But conventional metal pin and canine bone pin, as well as canine and ovine

bone pins, showed no significant differences with each other in no weeks of the study. Although, radiographic scores of both bone pins (OBP and CBP groups) were higher than conventional metal pin scores, but repeated measured analysis revealed that the conventional metal pin and the canine bone pin showed more similar trend of healing to each other over time. As, both mentioned groups (CMP and CBP) started to show significant healing at the 4th week. But conventional metal pin could not continue its optimum status longer than the 8th week and after that its radiographic scores differed non-significantly up to the 32nd week. Canine bone pin also stopped its optimum status at 12th week that was eight weeks sooner than the ovine bone pin. Ovine bone pin (OBP) group with the highest radiographic scores over the study time, started to heal significantly at the 2nd week and continued this optimal status till the 20th week; this showed that ovine bone pin could induce the bone healing better and sooner than the other studied implants.

Growth factors are essential for bone regeneration due to their effect on chemotaxis and differentiation of the osteoblasts and preosteoblastic cells; it is known that PDGF induces callus formation, TGF-X stimulates osteogenesis and inhibits bone resorption, IGF-I and VEGF induce bone formation, etc. Hypovascularity can compromise healing by affecting the osteoblast survival and local growth factors [19]. It seems that metal conventional pins can cause more and longer blood supply impairment [20]; while intra-medullary bone made pins provided the highest stability for humeral bone fracture in birds which fade after complete healing. Intra-medullary bone made pin and cerclage wire are the best technique to treat fractures in small and medium birds [21], although pinning is difficult in birds and may damage the nearby structures, but

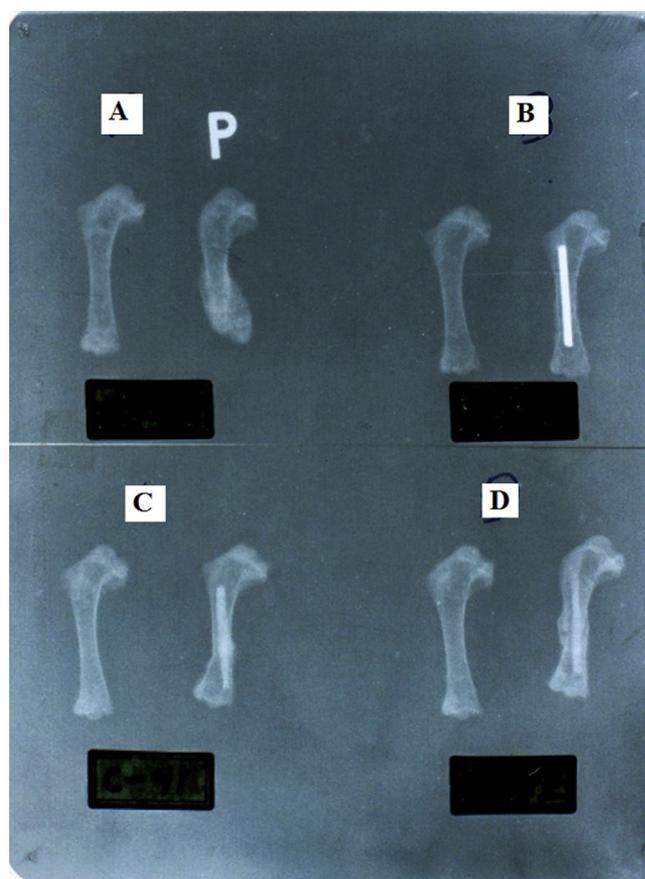


Fig. 5. Radiographs of the healed humeral bone during 32 weeks. A. The control group with no fixation, the healed humeral fracture (right) is shorter than the intact humeral bone (left) of the same pigeon. B. The healed humeral fracture fixed by conventional metal pin. C. The healed humeral fracture fixed by pins made of ovine long bone. D. The healed humeral fracture fixed by pins made of canine long bone.

provides good stability against bending forces for bone repair [4, 22].

In this study, none of the pigeon showed any interference in wound healing. In the control group, large amount of callus was formed in addition to cases of nonunion with fuzzy/hazy appearance because of existence of motion due to un fixed fracture ends [11, 18]; but in the implanted groups, there was a small amount of callus that was consistent with the primary union outcome. The important point here is the weight of the metal pins, which creates a state of imbalance in flight or non-flight position [4]. However, the bone pins made of sheep's or dog's long bones are free from most of the disadvantages and do not require to be removed due to gradual absorption, meanwhile providing a good stability throughout the repair process. Since the prepared bone pins from sheep's and dog's long bone were treated chemically, the immune responses was suppressed, that is why none of the birds in this study exhibited immune reaction, rejection or any other difficulty. These boney pins can also be called xenograft, heterograft or bone implants. However, histopathology and immunohistochemistry along with more advance flight analysis is suggested for further studies.

In conclusion the pins made of long bones of sheep and dog can be successfully used to treat the fractures of the pneumatic bones of birds, as they provide good stability, no tissue sensitivity, not need to be removed, absorbs gradually and helps with osteoconduction which is crucial part of bone repair process.

Declarations

Author contribution statement

Seifollah Dehghani Nazhvani: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Fatemeh Etemadi: Analyzed and interpreted the data; Wrote the paper.

Mehrdad Mohammadi: Conceived and designed the experiments; Performed the experiments.

Fatemeh Dehghani Nazhvani: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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