Effects of a 98% solution of glycerol or sterilization with ethylene oxide on FeLV in bone allografts and effects on bone incorporation of allografts in cats

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Objectives—To compare virucidal effects and bone incorporation properties of cortical bone allografts transplanted into specific-pathogen-free (SPF) cats. Allografts consisted of untreated bone from a SPF cat (negative-control group) and bone from 5 FeLV-infected cats that was subjected to sterilization with ethylene oxide (ETO), preservation with glycerol, or no treatment (positive-control group).

Sample Population—Bones from the aforementioned groups and twenty 8-week-old SPF cats (5 cats/group) implanted with an allograft from 1 of the aforementioned groups.

Procedure—After implantation, blood samples were collected weekly to monitor FeLV p27 antigen and antibody titers. Quantification of FeLV provirus was performed on blood samples at weeks 0, 4, and 8 and donor bone samples at time of implantation. Cats were euthanatized 8 weeks after transplantation, and graft sites were evaluated.

Results—All results for negative-control cats were negative. All ETO group cats had negative results for antigen and provirus in blood, whereas 1 cat had a low antibody titer. Although 3 ETO-treated allografts were positive for provirus, the DNA appeared denatured. One cat in the glycerol group had positive results for all tests in blood samples. All glycerol-preserved allografts were positive when tested for provirus. All results for positive-control group cats were positive. Differences in incorporation of bone grafts were not observed.

Conclusions and Clinical Relevance—Glycerol preservation of FeLV-infected bone allografts did not eliminate transmission of retrovirus to recipients. In contrast, ETO sterilization appeared to denature DNA and prevent infection. Treatments did not affect incorporation of bone grafts in young cats. (*Am J Vet Res* 2000;61:665–671)

The most common indication for use of cortical bone allografts in veterinary and human orthopedics is repair of large diaphyseal defects of long bones. The inconvenience of harvesting, processing, storing, and ensuring quality has restricted use of allografts in most veterinary practices. Preservation of bone allografts has been limited to ultra-low freezing or treatment with **ethylene oxide (ETO)**. Gamma irradiation also has been used to sterilize and reduce immunogenicity of allografts.[1] However, problems have been encountered with use of these sterilization procedures. One major disadvantage is that ETO may reduce bone induction and incorporation of host bone at the graft site.[2,3] Ethylene oxide also may negatively affect mechanical strength of the bone graft and have a toxic effect on fibroblast activity.[3-6] As an alternative to sterilization with ETO, canine cortical bone allografts stored in a 98% solution of glycerol appeared to have good incorporation into host bone, although quantification of this assessment was lacking.[7]

Viral and bacterial transmission are possible adverse outcomes when stored tissues are used for transplantation. Concerns that current freezing and storage practices may not be adequate to inactivate retroviruses have been substantiated by recent studies in animals in which scientists investigated viral transmission from retrovirus-infected transplanted allogenic cortical and cancellous bone and connective tissue grafts to recipient animals.[8,9] **Specific-pathogen-free (SPF)** cats had evidence of exposure to (positive results for antibody testing), or infection with (positive results for antigen testing), the retrovirus FeLV by 2 to 6 weeks after implantation of infected allogenic donor tissues that had undergone 1 or 2 freeze-thaw cycles before implantation. These studies were important, because they documented that retroviruses may be transmitted through transplantation of infected bone and connective tissues and that freeze-thaw cycles were inadequate to prevent transmission.[8,9]

Although some biological effects have been investigated,[2,3,6] we are not aware of studies that have examined virucidal properties of ETO on cortical bone allografts. Reports of studies from Europe[7] and South America[10] advocated use of a 98% solution of glycerol

for storage of bone and skin allografts. It is stated in other reports[11,12] that a 98% solution of glycerol is bactericidal and virucidal against enveloped and nonenveloped viruses, suggesting that efficacy against human immunodeficiency virus also may be possible. This latter claim was extrapolated from the observation that a 98% solution of glycerol appears to have in vitro virucidal effects on **herpes simplex virus, type 1 (HSV-1)** and poliovirus. If proven effective against pathogens, a 98% solution of glycerol would be a simpler, less toxic, and more cost effective alternative to sterilization with ETO.

The purpose of the study reported here was to compare antiviral properties and effects on bone incorporation of allografts obtained from FeLV-infected cats, using bones that were subjected to preservation with a 98% solution of glycerol, sterilization with ETO, or left untreated. We hypothesized that use of the 98% solution of glycerol would have similar virucidal effects as for ETO but would not be detrimental to incorporation of bone allografts in SPF cats.

Materials and Methods

Harvesting and preparation of bone allografts—Four weeks before implantation, intact sets of 5 metatarsal bones from an uninfected SPF cat and 3 groups of bones (n = 5 bones/group) from 5 cats (3 bones from each cat) infected with the Rickard strain of FeLV were aseptically harvested and stored fresh-frozen at –70 C. The Rickard strain was chosen to expand on previous studies that used this strain[8,9] and because most cats infected with this strain would not have signs of disease during the 8-week study period. Subsequently, bones harvested from the uninfected SPF cat (negative-control allografts) were thawed, placed separately into sterile plastic conical tubes, and refrozen at –70 C.

The 3 metatarsal bones from each of the FeLV-infected cats were allocated to 1 of 2 FeLV-infected treatment groups or to the positive-control group. After thawing, intact bones for group ETO were separately double-wrapped by use of heat-sealed plastic and sterilized with 100% ETO. After ETO sterilization, bone samples were aerated at 50 C for 12 hours (relative humidity, 30%) and refrozen at –70 C. Bones in the glycerol group were placed separately into conical centrifuge tubes that contained a 98% solution of glycerol, which were then stored in the dark at room temperature (22 C) for 4 weeks.[12] The positive-control group of bones did not undergo treatment and were placed separately into sterile plastic conical tubes and refrozen at –70 C. In addition, a small piece of the diaphyseal portion of the ulna of a FeLV-infected cat was aseptically harvested, placed into a sterile plastic tube, and stored at room temperature (22 C) for 4 weeks to test the effects of room temperature without treatment.

Animals for implantation—Twenty 8-week-old SPF cats were randomly allocated to 4 groups (n = 5 cats/group). Young cats were used because of their documented age-related susceptibility to FeLV infection.[13] The protocol was approved by a university animal care and use committee. Cats were housed separately in cages for 1 week to enable them to acclimate before implantation. Each cat of group 1 (negative-control group) was implanted with 1 cortical bone allograft obtained from the uninfected SPF cat. Each cat of group 2 (ETO group) received 1 ETO-sterilized cortical bone allograft obtained from a FeLV-infected cat. Each cat in group 3 (glycerol group) received 1 glycerol-preserved cortical bone allograft obtained from a FeLV-infected cat. Each cat in group 4 (positive-control group) received 1 untreated cortical bone allograft obtained from a FeLV-infected cat. Before and after implantation surgery, all cats were handled separately in accordance with a SPF protocol (coveralls, gloves, hats, masks, and shoe covers), and infected cats were handled last. Gloves were disinfected with bleach between cats within the same groups and were changed between groups of cats.

Surgical technique—Each cat was weighed, medicated with a combination of acepromazine maleate (0.03 mg/kg of body weight, IM) and butorphanol tartrate (0.1 mg/kg, IM), and given antibiotics (cefazolin; 22 mg/kg, IV) prophylactically. Anesthesia was induced by use of a mask; cats then were intubated, and anesthesia was maintained throughout surgery by use of halothane in oxygen. Fluids (lactated Ringer's solution; 10 ml/kg/h, IV) were administered throughout the anesthetic period.

The right or left ulna of each cat was randomly selected for implantation with a diaphyseal segment of bone allograft. A routine approach to the caudal ulna was performed,[14] and a periosteal elevator was used to elevate the periosteum from the exposed mid-diaphysis. A 1-cm mid-diaphyseal ostectomy was performed on the selected ulna, using Lembert rongeurs. During preparation of the cortical bone allograft, the open wound was lavaged and packed with 4 X 4 gauze sponges soaked with saline (0.9 % NaCl) solution. During surgery, the donor allograft was placed in sterile saline solution to equilibrate it to room temperature. The graft then was cut to a length of 1 cm, using a No. 10 scalpel blade. A 0.07-cm diameter piece of Kirschner wire was driven retrograde into the proximal segment of the ulna through the olecranon until the end of the pin apposed the edge of the proximal ostectomy site. After the bone graft was oriented and positioned in the ostectomy site, the Kirschner wire was driven normograde through the bone graft and into the distal metaphyseal segment. The Kirschner wire was cut close to the skin, using wire cutters, and the skin was pulled over the end of the wire. Fascia and skin were closed in a routine manner, using 4-0 polydioxanone and 4-0 nylon, respectively.

The remaining pieces of bone graft were saved for subsequent quantification of FeLV provirus. Radiographs were obtained after surgery to assess placement of the graft and Kirschner wire. Butorphanol (0.1 mg/kg, IM) was given to each cat after surgery as an analgesic. Cats were monitored for 8 weeks after transplantation to allow for virus replication within the host, then were euthanatized by use of an overdose of pentobarbital (87 mg/kg, IV).

Collection of blood samples—A blood sample was collected from each cat before surgery and placed into a tube containing EDTA. Samples were used to verify negative status for FeLV. Serial blood samples (3 ml) were collected weekly from each cat starting 2 weeks after surgery. When necessary, cats were sedated with a combination of ketamine hydrochloride (10 mg/kg, IM), midazolam (0.2 mg/kg, IM), and butorphanol (0.1 mg/kg, IM).

FeLV p27 antigen—An ELISA kit[a] was used to test for FeLV p27 antigen in plasma samples obtained at weeks 0 and 2 through 8. An ELISA microplate reader[b] was used to quantify antigen on the basis of **optical density (OD)**. The **sample-to-positive control ratio (S/P)** was calculated, using the following formula:

$$\frac{(OD\ of\ sample) - (OD\ of\ negative\text{-}control\ sample)}{(OD\ of\ positive\text{-}control\ sample) - (OD\ of\ negative\text{-}control\ sample)}$$

Calculated S/P values of ≥ 0.1 were considered to be positive results.[15]

FeLV antibody—Antibody titers to **feline oncornavirus cell membrane-associated antigen (FOCMA)** were measured

in plasma samples at weeks 0 and 2 through 8, using a live-cell immunofluorescence assay, as described elsewhere.[16] Antibody titers of ≥ 1:16 were considered positive.

Extraction and quantification of DNA—The DNA was extracted from buffy coats of anticoagulated blood samples collected from each cat at weeks 0, 4, and 8, using a DNA extraction kit.[c] Remaining sections of bone graft obtained at the time of implantation, as well as the bone sample stored untreated at room temperature, were ground to powder, using a freezer-mill.[d] The DNA was extracted from each sample of bone powder by use of a phenol-chloroform technique[17]; extracted solutions were electrophoresed through a 1% agarose gel and stained with ethidium bromide.

The DNA extracted from blood and bone samples was quantified, using a DNA fluorometer.[e] Samples of DNA were digested with the restriction endonuclease *Eco*R1, electrophoresed through a 3% agarose gel, and stained with ethidium bromide to confirm detection and uniformity of DNA in each sample. Digested DNA samples were stored at 4 C in sterile microfuge tubes.

Although all bone grafts underwent the same process of DNA extraction, a low quantity of DNA was obtained from ETO-sterilized bone grafts, impeding quantification of FeLV provirus. Consequently, larger bone samples were collected from the ulna of the corresponding limb of each donor cat. These samples were subjected to ETO sterilization, DNA extraction, and **quantitative polymerase chain reaction (QPCR)**.

FeLV provirus—A QPCR assay was used to quantify FeLV proviral DNA in 100 ng of digested DNA extracted from blood and bone samples. Negative and positive calibration standards were assayed in parallel with test samples. Other PCR assays for detection of FeLV provirus have been reported.[15,18]

Radiographic and histologic evaluation of implanted limbs—After recipient cats were euthanatized, each implanted ulna was harvested and placed in neutral-buffered 10% formalin. High-resolution radiographs were taken of each harvested ulna, using a faxitron[f] and kodalith-ortho film.[g] Each ulna then was decalcified, using 5% nitric acid, and sections were prepared and stained with H&E for histologic examination. Subjective histologic analysis was performed to evaluate host-graft interfaces, healing of the bone graft, and incorporation of the bone graft by the host. Analysis was concentrated at the proximal and distal host-graph interfaces. Evaluations included periosteal overgrowth, amount of dead graft bone (identified as empty lacunae), amount of incorporation of host bone into graft bone, and number of inflammatory cells.

Results

Cats—At the time of implantation, cats were 6.4 to 8.0 weeks old (mean, 7.2 weeks) and weighed between 0.45 and 0.90 kg (mean, 0.68 kg). At the time they were euthanatized, cats weighed between 1.36 and 1.80 kg (mean, 1.52 kg).

Surgical outcome—All cats recovered from anesthesia without complications. Nine cats had bone allografts implanted in the left ulna, and 11 cats had bone allografts implanted in the right ulna. On the basis of examination of radiographs taken immediately before and after surgery, all bone grafts had good apposition within the ostectomy site, and the position of the Kirschner wire was satisfactory in all cats, except for 2 (1 in group ETO and 1 in group glycerol). The pin was placed medially to the distal portion of the ulna in the cat in group ETO. That cat recovered well and was not lame. The cat in group glycerol was reanesthetized, and the pin was repositioned because it had passed into the carpus. That cat and 2 other cats were lame in the affected limb for 1 week after surgery but subsequently used the limb appropriately. Complications with nonunion or malunion were not observed, and all grafts were incorporated into host bone. All 20 cats eventually used the grafted limb well. One cat in the positive-control group had early signs of carpal valgus at week 2; this condition slowly progressed during the duration of the study. One cat in the glycerol group developed a seroma at the olecranon area of pin placement at week 7, which resolved during the subsequent few days. Another cat in the glycerol group developed acute lameness of the implanted limb at week 8. Manipulation of the limb did not elicit signs of pain in that cat; however, a transverse fracture of the radius and ulna at the distal graft site was discovered when the limb was examined after the cat was euthanatized. We hypothesized that fractures of the radius and implanted ulna were attributable to trauma from the cage, because adequate incorporation of the graft was evident at proximal and distal host-graft interfaces, and a bent Kirschner wire was evident on radiographs of the limb.

Health status of cats—Six cats had transient vomiting or diarrhea during the study (2 cats from the negative-control group, 3 from the ETO group, and 1 from the positive-control group). Five cats had transient sneezing or coughing (1 from the negative-control group, 1 from the ETO group, and 3 from the positive-control group). All but 1 of the cats were in good general health throughout the study. One of the cats in the positive-control group was given antibiotics because of a respiratory tract infection at week 4. That cat initially responded to treatment but relapsed and died at week 6. We hypothesized that the cat was immunocompromised as a consequence of FeLV infection.

FeLV p27 antigen—All cats had negative results when tested for FeLV p27 antigen just before surgery (week 0). Cats in the negative-control and ETO groups had negative results for FeLV p27 antigen throughout the entire 8-week study. One cat from the glycerol group had positive results for FeLV p27 antigen at weeks 5 and 6, then reverted to negative results at weeks 7 and 8. All positive-control cats had positive results for FeLV p27 antigen at weeks 2 and 3. Two of these cats reverted and had negative results for the remainder of the study, whereas the other 3 positive-control cats had positive results throughout the remainder of the study, including the cat that died at week 6 (**Table 1**).

FeLV antibody—All negative-control cats were seronegative to FOCMA at week 0 and remained seronegative for the remainder of the study (**Table 2**). One of the cats in group ETO had low titers (1:16) from weeks 3 through 8, but all other cats in that group were seronegative to FOCMA. One cat in the glycerol group had moderate antibody titers to FOCMA (1:32
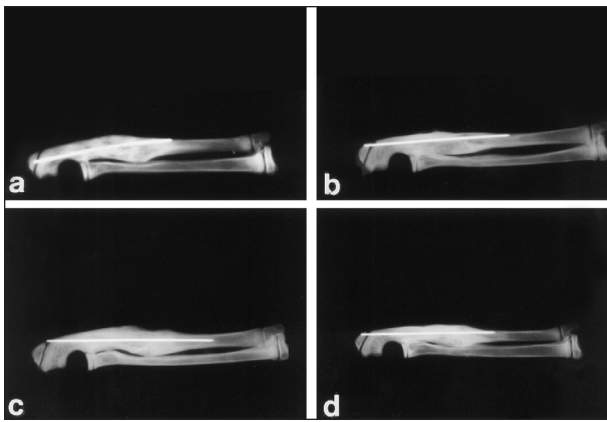
Figure 1—High-resolution radiographs of the radius and ulna from 4 cats 8 weeks after implantation with a cortical bone allograft. Moderate callus formation was evident around the graft site in all cats. a = Negative-control group. b = Positive-control group. c = Glycerol-treated group. d = Ethylene oxide-treated group.
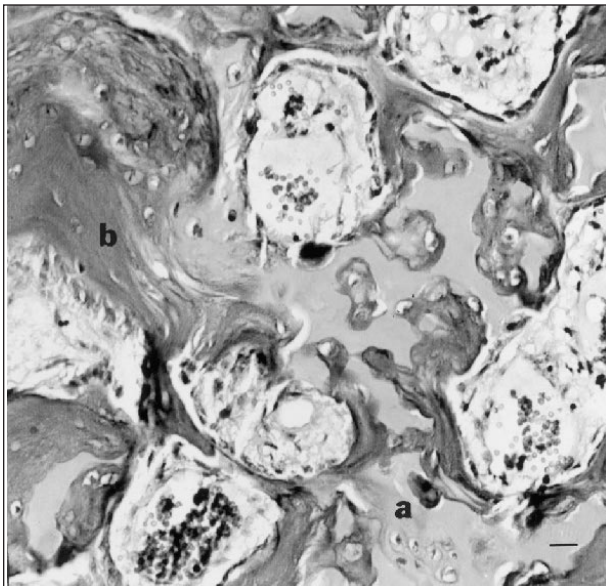

Figure 2—Photomicrograph of a section of bone from the host-graft interface in the ulna of a cat that received a cortical bone allograft. Dead bone graft can be seen as empty lacunae (a) surrounded by new woven host bone (b). H&E stain; bar = 20 μm.

to 1:128) at weeks 6 through 8. Four of the cats in the positive-control group had positive titers (range, 1:16 to 1:128) at week 2. All cats in the positive-control group had moderate to high antibody titers to FOCMA from week 3 through the remainder of the study (range of titers at week 8, 1:128 to 1:2,096).

FeLV provirus in blood samples—All cats had negative results when tested for FeLV proviral DNA by QPCR before surgery (week 0). All cats in the negative-control and ETO groups had negative results for FeLV proviral DNA throughout the study. One cat in the glycerol group had positive results for FeLV proviral DNA at weeks 4 (257 copies) and 8 (8,729 copies), whereas all other cats in the glycerol group had negative results for FeLV provirus. All positive-control cats

Table 1—Number of cats with positive results when tested for FeLV p27 antigen after implantation with a cortical bone allograft in the mid-diaphyseal portion of the ulna

	Week after implantation							
Group	0	2	3	4	5	6	7	8
Negative-control (n = 5)	0	0	0	0	0	0	0	0
ETO (n = 5)	0	0	0	0	0	0	0	0
Glycerol (n = 5)	0	0	0	0	1	1	0	0
Positive-control (n = 5)	0	5	5	3	2	3	2*	2*

*n = 4.
 Negative-control group = Untreated allografts from uninfected cats. ETO group = Ethylene oxide-treated allografts from FeLV-infected cats. Glycerol group = 98% Solution of glycerol-treated allografts from FeLV-infected cats. Positive-control group = Untreated allografts from FeLV-infected cats.

had detectable FeLV proviral DNA at weeks 4 (range, 2,772 to 468,021 copies; mean, 204,428 copies) and 8 (range, 661 to 521,192 copies; mean, 243,178 copies). Three cats in the positive-control group had decreased proviral loads, and 1 cat had an increased proviral load from weeks 4 to 8. The remaining cat in the positive-control group died at week 6 (**Table 3**).

Agarose gel electrophoresis—Following agarose gel electrophoresis and ethidium bromide staining, DNA from bones of negative-control, glycerol-treated, and positive-control groups appeared intact and had distinct bands. In contrast, DNA from the ETO group did not have distinct DNA bands; it was smeared throughout the length of the gel, appearing denatured and suggestive of reduction or elimination of intact virus or provirus.

FeLV provirus in donor bone—All negative-control allografts implanted into cats in the negative-control group had negative results when tested for FeLV provirus. Of 5 (range 0 to 20,318 copies; mean ± SEM, 5,733 ± 3,400 copies) ETO-treated bones, 3 had positive results for FeLV proviral DNA (range, 2,157 to 20,318 copies; mean = 9,555 copies). All 5 glycerol-treated bones had positive results for FeLV proviral DNA (range, 4,459 to 80,464 copies; mean ± SEM, 47,120 ± 11,690 copies). In contrast, blood samples from only 1 of the recipient cats in the glycerol group had positive results when tested for FeLV proviral DNA. All untreated positive-control bones had positive results for FeLV proviral DNA (range, 7,322 to 87,113 copies; mean ± SEM, 49,459 ± 15,760 copies), as did recipient blood samples. The untreated bone graft stored at room temperature for 4 weeks had less FeLV proviral DNA (3,750 copies) than glycerol-treated or untreated bones (Table 3).

Radiography—High-resolution radiographs taken of implanted limbs after cats were euthanatized revealed moderate callus formation at proximal and distal host-graft interfaces in all cats (**Fig 1**). Host bone appeared incorporated into graft bone at all host-graft interfaces, and graft bone was almost indistinguishable in most cats. Subjectively, differences in healing were not observed among groups.

Histologic examination—Good incorporation of the donor graft by host bone was observed histologically; we did not detect appreciable differences among groups. Active remodeling and incorporation was

Table 2—Number of cats with antibody titers to feline oncornavirus cell membrane-associated antigen ≥ 1:16* in blood samples following implantation with a cortical bone allograft in an ulna

Group	Week after implantation							
	0	2	3	4	5	6	7	8
Negative-control (n = 5)	0	0	0	0	0	0	0	0
ETO (n = 5)	0	0	1 (1:16)	1 (1:16)	1 (1:16)	1 (1:16)	1 (1:16)	1 (1:16)
Glycerol (n = 5)	0	0	0	0	0	1 (1:32)	1 (1:128)	1 (1:128)
Positive-control (n = 5)	0	4 (1:16–1:128)	5 (1:64–1:128)	5 (1:64–1:128)	5 (1:64–1:512)	5 (1:128–1:2096)	4† (1:128–1:1024)	4†(1:128–1:2096)

Values reported indicate number of cats (range of titers).
*Titers ≥ 1:16 were considered positive. †n = 4.
See Table 1 for key.

Table 3—Results of a quantitative polymerase chain reaction test to detect the number of copies of FeLV provirus in blood and bone graft samples of cats that received a cortical bone allograft in an ulna

Group	Copies of FeLV provirus			
	Blood samples (week after implantation)			
	0	4	8	Bone samples*
Negative-control (n = 5)	0	0	0	0
ETO (n = 5)	0	0	0	3 (5,733 ± 3,400)
Glycerol (n = 5)	0	1 (51)	1 (1,746)	5 (47,120 ± 11,690)
Positive-control (n = 5)	0	5 (204,428 ± 107,716)	4† (243,178 ± 140,668)	5 (49,459 ± 15,760)
Room temperature‡ (n = 1)	NA	NA	NA	3,750

Values reported indicate the number of cats or bones with positive numbers (mean ± SEM) of copies for FeLV provirus.
*Bone samples were obtained from sections of grafts implanted into recipient cats. †n = 4. ‡Results (recorded as No. of copies) for a bone sample stored at room temperature for 4 weeks.
NA = Not applicable.

detected in all bones along proximal and distal host-graft interfaces. At the graft interfaces, dead graft bone was surrounded by new woven host bone. Bone grafts were identified by empty lacunae within bone surrounded by new woven host bone (**Fig 2**). Moderate periosteal proliferation was observed bridging the host-graft interfaces. Moderate infiltration of neutrophils was evident at the graft sites, but differences in inflammatory or toxic changes were not observed among groups.

Discussion

Various techniques have been reported for preservation and sterilization of canine cortical bone allografts.[1,4,6,7] However, their use by most veterinary practitioners has been restricted by the cost and inconvenience of allograft collection, processing, and storage. Typical methods used include sterile harvesting of the graft followed by low-temperature storage (–20 to –40 C) or lyophilization (freeze-drying). Frozen bone grafts have a shelf life of only 6 to 12 months. Freeze-drying grafts has the advantage of allowing storage at ambient temperature, but this technique reportedly decreases mechanical strength of bone.[1]

Secondary sterilization methods can be used to eliminate the need for aseptic harvesting of bone grafts, thereby reducing cost and increasing convenience. An effective sterilization technique uses γ–irradiation for deep penetration of thick tissues, but it requires instruments that may not be available in most veterinary hospitals. In addition, high doses of irradiation have been associated with decreased osteoinductive and biomechanical properties of bone grafts.[1] Ethylene oxide has been widely used for sterilization and reportedly is effective in removing surface contamination, but it

decreases bone induction and incorporation properties and reduces mechanical bone strength.[2-6] An optimal storage and sterilization technique would eliminate the need for special equipment, sterile harvesting of grafts, or both.

Reports of viral transmission in humans after transplantation of soft-tissue or bone grafts have raised concerns regarding sterilization and storage of these grafts.[19-22] Although ETO reportedly is an effective bactericidal agent, its virucidal properties in tissues have not been documented. In contrast, virucidal activity of an 85% solution of glycerol against HSV-1 and polioviruses has been reported.[11] Soft-tissue grafts stored in an 85% solution of glycerol at 20 C had complete inactivation of HSV-1 and poliovirus after 8 and 22 days of storage, respectively. Because that was an in vitro study, data were not available regarding the effects of transmission of these viruses in an in vivo system. Investigations of the virucidal properties of glycerol on transplanted bone grafts have not been described. However, treatment of canine femoral cortical bone allografts with a 98% solution of glycerol at ambient temperature was adequate for storage and resulted in good healing when a bone plate was used for stabilization.[7] Complete periosteal bridging was seen at the graft sites with a continuity of cortices at the host-graft interfaces 90 days after graft implantation.

In the study reported here, results of ETO sterilization were promising, because all recipient cats had negative results when tested for viral antigen and provirus, and we did not detect evidence of viral transmission. Although 3 of 5 ETO-treated donor bone grafts had positive results when tested for FeLV provirus, none of the recipient cats in this group became infected after transplantation. Lack of viral

transmission to recipients of ETO-treated bone grafts may be attributable to a decrease in the infectious viral load. Additionally, the 3 bones from the ETO group that had positive results (mean, 9,555 copies) had less FeLV provirus than bones for the glycerol (mean, 47,120 copies) or positive-control (mean, 49,459 copies) groups (Table 3).

Antibody titers to FOCMA ≥ 1:16 may result from exposure to infectious virus or, possibly, viral antigens. A low-positive antibody titer to FOCMA was detected in 1 cat in the ETO group, despite lack of viral antigenemia. This may have resulted from a viral antigen in the bone graft stimulating an antibody response in the recipient, despite lack of intact infectious virus. Alternatively, antigen concentrations may have been too low to detect a positive reaction in blood samples.

Cats in this study did not have evidence of ETO toxicosis. Toxic byproducts of ETO sterilization include ethylene glycol and ethylene chlorhydrin. Apart from the toxic effects of these ethylene oxide residuals, another possible explanation for reduced incorporation may be ETO-induced alkylation of amino acids.[3] In the study reported here, equivalent bone incorporation was observed in cats in the ETO group, compared with cats in the untreated negative- and positive-control groups. Although there were undoubtedly toxic byproducts, lack of difference in incorporation in this model may be partly attributable to the young age of the cats and their high propensity for healing. A study that uses adult cats would likely reveal differences in incorporation.

Transmission of FeLV to 1 of the cats in the glycerol group may have been associated with immunocompetence of the recipient, infectious virus titer of the graft, or both. Because all donor bones in the glycerol group had more FeLV provirus (mean, 47,120 copies) than the untreated bone stored at room temperature for 4 weeks (3,750 copies), it is possible that the 98% solution of glycerol enhanced viral preservation. Unfortunately, infectivity could not be assessed in the untreated bone, because it was not implanted in a cat. Despite the fact that ambient storage of untreated FeLV-infected bone appeared to decrease viral load, the resultant putrefaction precludes its use.

Donor bones in the positive-control group had the highest amount of FeLV provirus (mean, 49,459 copies). All cats in the positive-control group had positive results when tested for FeLV p27 antigen 3 weeks after transplantation. Although 2 cats in the positive-control group subsequently had negative results for FeLV p27 antigen (Table 1), these cats had positive results for FeLV provirus at weeks 4 and 8 (Table 3). Transient antigenemia has been reported in cats with natural FeLV infection or infection resulting from experimental implantation of FeLV-infected bone and connective tissues.[8,9] However, investigators in those studies did not assess FeLV status by use of the more sensitive quantitative PCR technique. All cats in the positive-control group were seropositive to FOCMA, confirming exposure to FeLV. Furthermore, all cats had positive results for FeLV provirus, documenting infection with FeLV.

Whole, intact FeLV provirus or smaller amplifiable regions of FeLV provirus may be detected in DNA samples analyzed by QPCR. Detection of the small segment of provirus amplified by QPCR does not establish infectivity of donor bone grafts. In contrast, detection of provirus or antigen in blood after allotransplantation of infectious bone establishes transmissibility of the retrovirus. The fact that all cats in the positive-control group had positive results when tested for FeLV antigen and provirus in blood samples confirms that freezing does not effectively impair viability of the retrovirus or prevent transmission after implantation.

Results of the study reported here documented that ETO sterilization appeared to denature DNA and had effective virucidal activity against the retrovirus FeLV. In contrast, use of a 98% solution of glycerol was inadequate for viral sterilization of cortical bone allografts. Comparison of the virucidal effects of glycerol-preserved grafts with that for untreated positive-control grafts did not reveal a reduction in the quantity of amplifiable FeLV provirus in donor grafts treated with a 98% solution of glycerol. Although transmission of FeLV was decreased in recipients of glycerol-treated bone grafts, suggesting decreased infectivity, a 4-week duration of glycerol treatment for bone allografts cannot be recommended for virucidal sterilization. Additional studies may be warranted to examine the effect of prolonged (eg, 6 months) glycerol treatment on virus-infected bone grafts.

Histologically, we did not detect differences in incorporation at the host-graft interface among groups. It may be concluded from this study that bone allografts sterilized by the use of ETO or a 98% solution of glycerol had comparable incorporation of host bone, compared with that for untreated-control groups in this model that used young cats. However, ETO sterilization had superior virucidal activity against the retrovirus FeLV.

Ethylene oxide sterilization appeared to denature DNA and abrogate transmission of FeLV infection. However, quantities of provirus detected by QPCR in ETO-treated donor bone grafts were reduced but not eliminated. Additional studies to determine whether provirus in ETO-treated bone was intact infectious virus or smaller noninfectious segments of proviral DNA are warranted. Lack of transmission of FeLV to recipients of ETO-treated bone grafts suggests that the veterinary community may be cautiously optimistic regarding the safety and efficacy of this widely available treatment.

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