INTRODUCTION
It is difficult to establish the efficacy of disease modifying osteoarthritis (OA) drugs because OA progression is highly variable and occurs slowly over decades. Animal models of OA are useful because OA progresses rapidly in a period of months, allowing efficient and economical evaluations. A common animal model of OA is the Pond-Nuki or anterior cruciate ligament transection (ACLX) model. Disease modifying agents can be difficult to study using this model because biomechanical instability of the knee leads a series of repeated, superimposed injuries. This makes the rate of OA progression variable and the lesions so severe that they may be difficult to control with therapeutic drugs or biologics. Our goal was to determine if the protective effect of a disease modifying OA drug could be interpreted more clearly in a slowly progressive OA model.

METHODS
All procedures were carried out in accordance with The Canadian Council on Animal Care. This study compared the contusive impact (CI) model of osteoarthritis against the anterior cruciate ligament transection (ACLX) OA model using 24 Foxhounds randomly allocated to four experimental groups. In the first group (CI model-no treatment) OA was induced in one knee joint of five dogs by application of a 15 MPa impact to the weight bearing region of the medial femoral condyle through a small arthrotomy using a spring loaded device. A second group (n=7) received contusive impact injury and polysulfated glycosaminoglycans (Adequan® 4.4 mg/kg i.m. twice weekly for four weeks postoperatively (CI+Adequan). In the ACLX model, the third group was composed of 5 dogs that underwent ACL transection (ACLX-no treatment) and a fourth group of ACLX dogs (n=7) received Adequan®. All dogs were exercised twice daily and scoring of lameness and gait deficits was done by two investigators each week. After 12 weeks all dogs were sacrificed, knee radiographs were taken and macroscopic scoring of joint surface abnormalities was conducted. Cartilage sulfated glycosaminoglycan concentration, biomechanical indentation indentation time constants, and release of nitric oxide and sulfated glycosaminoglycans from LPS stimulated cartilage explant tissue cultures were measured. A detailed histopathology scoring system that considers lesion grade and extent was applied to four sites: the medial femoral condyle, medial tibial plateau and lateral femoral condyle, and the contralateral unoperated medial femoral condyle. All assessments were done by personnel who were blind to the group allocation of the dogs. Statistical comparisons were made between control and treatment groups within each model using parametric and non-parametric statistics depending on the type of data, followed by post-hoc tests and corrections for multiple comparisons as required.

RESULTS
Weekly lameness scores were not significantly different between OA and OA + treatment groups in either model but ACLX dogs had lameness that persisted throughout the study whereas CI model dogs demonstrated minimal pain and dysfunction after 3 weeks postoperatively. Radiographic scores indicated a trend toward more periosteal proliferation (osteophytes) in the ACLX+Adequan group (p<.06). Mild cartilage discoloration and loss of surface sheen was present in the medial femoral condyle of both CI model groups whereas extensive, moderate to severe cartilage abnormalities, including erosions, were found at multiple sites in both ACLX groups. Macroscopic scoring of the joint surfaces revealed significant differences between the CI and CI+Adequan groups though such lesions were very mild and difficult to detect in both groups. In the ACLX model, there was evidence of protection against cartilage lesions in the ACLX+Adequan group (p<.001).

Cartilage sGAG concentration was improved by Adequan administration in the CI model at the medial femoral condyle (p<.06), lateral condyle (p<.01) and contralateral condyle (p<.05) but not the tibia. In the ACLX model, Adequan preserved sGAG cartilage concentration at the lateral condyle (p<.05) and contralateral condyle (p<.01).

There were no differences in NO production between groups in either model. Unexpectedly, Adequan-treated groups lost more sGAG than control groups in both models. In the CI+Adequan group there was significantly more sGAG released before LPS exposure (p<.03, ANOVA) and a similar trend (p<.08) after exposure. The ACLX+Adequan group sGAG loss from explants was significantly greater before (p<.01) and after (p<.001) exposure to LPS.

DISCUSSION
As in previous studies, the ACLX model produced clear macroscopic, biomechanical biochemical and histological lesions in twelve weeks whereas milder, more focal lesions result from the CI model. The structural changes to the collagenous network of cartilage in the ACLX model were severe enough to be irreversible whereas the CI model produced hypertrophic rather than clear degenerative changes at this time point. In our opinion, the CI model leaves more potential for reversibility and measuring therapeutic effects because the initial impact injury improved and does not progress centrifugally when a chondroprotective drug was used. This was unexpected because direct mechanical injury in the CI model and instability leading to subluxation in the ACLX model, can make it difficult to demonstrate treatment effects in the medial femoral condyle. Nevertheless, tissue sGAG concentrations, a key indicator of healthy cartilage metabolism, and histological scores were improved in the contusive impact model. We acknowledge that larger experimental groups are needed; post-hoc sample calculations indicate that groups of ten animals are needed in both models for adequate statistical power.

We expected LPS to stimulate additional release of sGAG from cartilage explants derived from the medial femoral condyle however osteoarthritic cartilage may be so matrix-depleted that this is not possible. Hence, the stimulation of sGAG release from Adequan-treated cartilage explants may be a feature of increased sGAGs available to undergo LPS-stimulated enzymatic digestion. In the present study, the entire matrix may have been depleted, resulting in low sGAG release from CI model-no treatment and ACLX-no treatment groups.

The CI model of OA caused less lameness and disability in dogs making it a more humane alternative than ACL transection. In some regards, this model appears to offer more potential for measuring therapeutic effects of osteoarthritis modifying drugs because lesions are potentially reversible. This would allow serial assessments at longer time points. By contrast, any therapeutic benefits in the ACLX model would be abolished by development of several, erosive osteochondral lesions resulting in a convergence of control and treated groups.

REFERENCES

Acknowledgements: Deb McWade, Nicole Kudo, Michelle Beaudoin, Mary Martini & staff for technical assistance. CI Model development was assisted by a grant from the Canadian Arthritis Network.