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ESTUDIO EXPERIMENTAL DE LA ARITCULACIÓN DE RODILLA CANINA SOMETIDA A TRANSLACIÓN CRANIAL DE LA TIBIA: COMPARACIÓN ENTRE LA RODILLA INTACTA, CON LCA DEFICIENTE Y CON LA ATT.

Experimental Study of Canine Stifle Joint Stiffness Under Cranial Tibial Translation: Comparison Between the Intact, the ACL-Deficient and the TTA Knee

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RESUMEN

El avance de la tuberosidad tibial (ATT) constituye una técnica usada para reparar la articulación fémoro-tibio-patelar (de la rodilla) después de una lesión de rotura del ligamento cruzado cranial (LCCr). El objetivo de este estudio fue comparar la rigidez (es decir, la fuerza entre desplazamiento) de la articulación de la rodilla intacta respecto a la misma después de seccionar el LCCr y de aplicar la técnica de reparación del ATT. El desplazamiento craneal de la tibia respecto del fémur se simuló en ensayos experimentales mediante el desplazamiento caudal del fémur respecto de la tibia fijada. Esta fuerza tangencial fue aplicada en 8 articulaciones fémoro-tibio-patelares (*in vitro*) obtenidas de perros adultos con un rango de pesos de 25 a 30 kg. Los valores de fuerza y desplazamiento registrados se usaron para calcular y comparar rigideces (en Newton/mm). Después de seccionar el LCCr, el desplazamiento incrementó 3,8 veces y la rigidez decreció 76% respecto a la articulación de la rodilla intacta. El valor de rigidez en la articulación de la rodilla sana no fue alcanzado después de la intervención con el dispositivo de la ATT, pero el desplazamiento se redujo a la mitad y la rigidez se duplicó, comparada con la articulación de la rodilla con el LCCr deficiente. La técnica de la ATT no frena complentamente el desplazamiento craneal de la tibia respecto al fémur, sin embargo, el valor de la rigidez de la articulación de la rodilla reparada duplica el valor de la rodilla con LCCr deficiente.

Palabras clave: Ligamento; biomecánica; articulación de la rodilla canina; prótesis ortopédicas; avance de la tuberosidad tibial

ABSTRACT

The tibial tuberosity advancement (TTA) is used to repair the knee after anterior cruciate ligament (ACL) rupture. The aim of this study was to compare the stiffness (i.e. force per displacement) of the intact knee to that of the same knee after ACL section and after applying the TTA repair technique. The cranial displacement of the tibia relative to the femur was simulated in experimental trials by applying a caudal displacement of the femur on a fixed tibia. This tangential force was applied on 8 femorotibial joints (*in vitro*) obtained from adult dogs weighing between 25 and 30 kg. The recorded force and displacement values were used to calculate and compare stiffnesses (in Newton/millimeters). After sectioning the ACL, the displacement was 3.8 times higher and the stiffness decreased by 76% compared to the intact knee. The healthy knee stiffness value was not reached after repairing the knee with the TTA device, but displacement was halved and stiffness doubled compared to the ACL-deficient knee. The TTA device does not completely stop the anterior displacement of the tibia relative to the femur; however, the stiffness value of the repaired knee doubled that of the ACL-deficient knee.

Key words: Ligament; biomechanics; canine stifle joint; orthopaedic plates; tibial tuberosity advancement

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INTRODUCTION

Cranial cruciate ligament (CrCL) pathology is the most common cause of canine (C*anis lupus familiaris*) stifle lameness [13]. Rupture of the CrCL leads to abnormal craniocaudal motion of the tibia and excessive internal rotation of the stifle joint, which leads to progressive osteoarthritis [2, 5, 21]. Surgical techniques to re-stabilize the joint, with either static or dynamic repairs were performed to neutralize the tibiofemoral shear forces in a CrCL deficient knee [11, 14, 16]. Tibial Tuberosity Advancement (TTA) is based on a model analysis of the human knee, which characterizes joint forces acting on the knee in a weight-bearing position, and has been successfully adapted for its use in dogs [9, 11, 18, 19]. The biomechanical principle of the method consists of the neutralization of the cranial tibial thrust force by placing the patellar tendon perpendicular to the tibial plateau [20]. The technique promotes less alteration to the intraarticular and periarticular components, and therefore leads to superior clinical results [20]. The TTA procedure aims to advance the tibial tuberosity cranially in order to maintain a patellar tendon angle (PTA) of less than, or equal to 90° during weight-bearing, therefore stabilizing a CCL-deficient stifle [3, 17, 22]. After the CrCL rupture, the tibia tends to move cranially and the knee suffers a loss of stiffness, in other words, the laxity increases. It is known that TTA stops tibial cranial displacement [1, 4, 7, 9] but there are no previous studies that quantify the improvement of knee stiffness after reparation with the TTA technique.

The study aims to assess and compare knee stiffness in three cases: intact canine knee, CrCL-deficient knee, and knee repaired using a TTA system. The concept of knee stiffness (i.e. the relation between load and displacement - Newton/mm) is used instead of laxity (only indicating displacement - mm). They are opposing concepts, since at lower laxity, greater knee stiffness. Stiffness is calculated using the displacement and force data provided by the inductive transducers and the force sensor. The objectives of this study were to compare the stiffness of the complete anatomical knee to the CrCL-deficient knee and the TTA-repaired knee; to know the percentage of loss of stiffness in the knee after CrCL excision; and to know the percentage of stiffness increase in the knee after repairing it using the TTA system.

MATERIALS AND METHOD

The cranial displacement of the tibia relative to the femur is simulated in experimental trials by applying a caudal displacement of the femur on a fixed tibia (FIG. 1). Due to the relative motion that exists between femur and tibia, the caudal femoral displacement on a fixed tibia is equivalent to the cranial displacement of the tibia on a fixed femur. The knee specimens were fixed to a testing system designed and constructed in the laboratory (FIG.2).
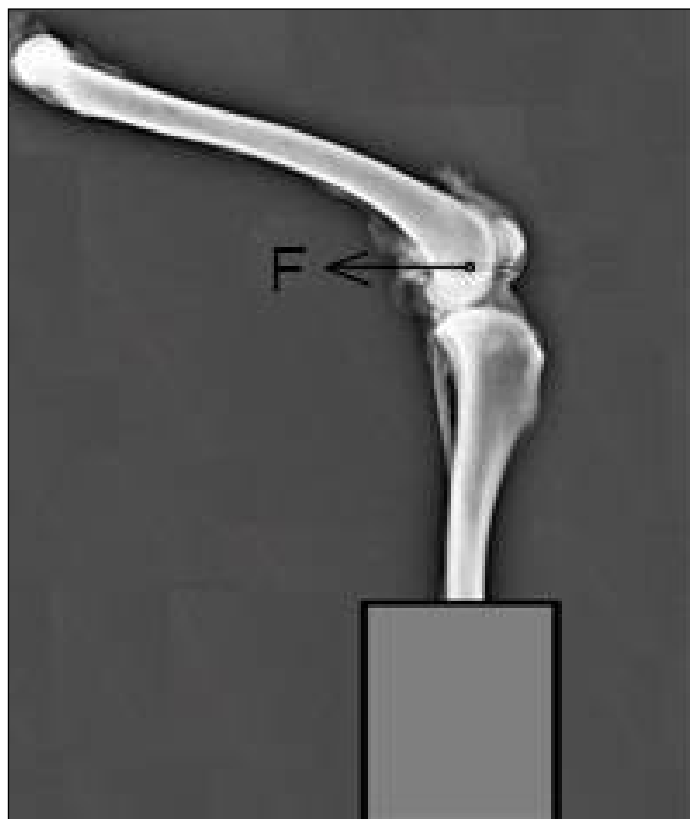

FIGURE 1. APPLICATION OF A CAUDAL FORCE ON THE FEMUR IN A FIXED TIBIA TO SIMULATE THE CRANIAL DISPLACEMENT OF THE TIBIA (DRAWER EFFECT).
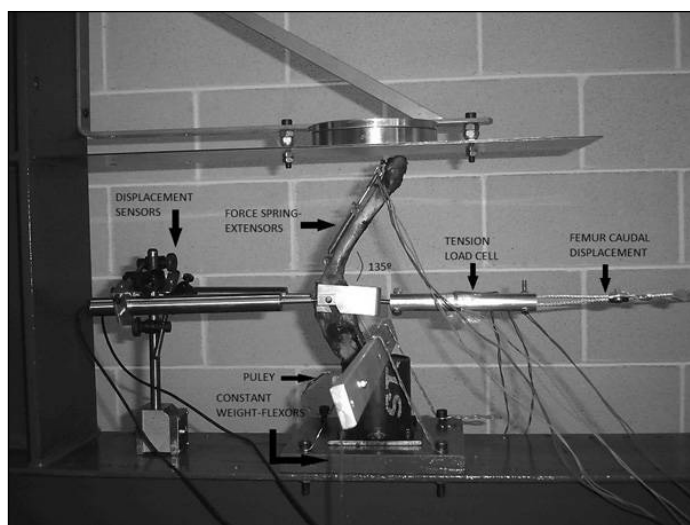

FIGURE 2. SPECIMEN ASSEMBLY PRIOR TO THE TRIALS AND MEASURING SYSTEMS (ON THE LEFT OF SPECIMEN DISPLACEMENT SENSORS AND ON THE RIGHT, FORCE SENSOR).

Specimen preparation

The tested specimens were 8 canine cadaver fémoro-tibial-patelar joints. The specimens were obtained from canine cadavers that were sacrificed by others pathologies with their owner's consent. The specimens' weights ranged from 30 to 35 kilograms (kg) which radiologically required a 9 mm advancement. The bones were disarticulated at the hip joint (articulatio coxae), preserving the femoral head, and the tibia was sectioned distally, on its distal third. All soft tissues were removed except for the patella and patellar tendon and the quadriceps muscle, the stifle joint capsule, the collateral ligaments and the sesamoid bones of the gastrocnemius muscle. The specimens were frozen[1] at -18 °C until it was time to perform the trials. The fémoro-tibial-patelar joint was fixed by adding a composite material in a metal container that embeds the specimen when hardened.

A 2 mm hole was drilled at the level of the bone bridge that links the trochanter to the femoral head, and another one in the patella; these two orifices were used to anchor a spring whose mission was to simulate the force exerted by the quadriceps muscle. The force of the quadriceps tendon is, according to Shahar and Bank-Sills [15], approximately equal to 48.5% of the animal's weight. Since the trials were performed on specimens free of muscles and tissues a reduction factor was applied. For a 30 kg dog, the tendon was simulated with a spring that exerted a force of 15 N. Two 3.5 mm screws were also inserted at the level of both sesamoid bones with a washer to fasten the cables simulating the calcaneal tendon (Achilles tendon) charges. According to Shahar and Bank-Sills [15], the total strength of the muscles that are attached to the calcaneal tendon is 29.09% of the dog's weight. A tendon was simulated for a 30 kg animal and, after applying a reduction factor, a force of 9.81 N was applied via two standardized 0.5 kg weights (FIGS. 2 and 3).
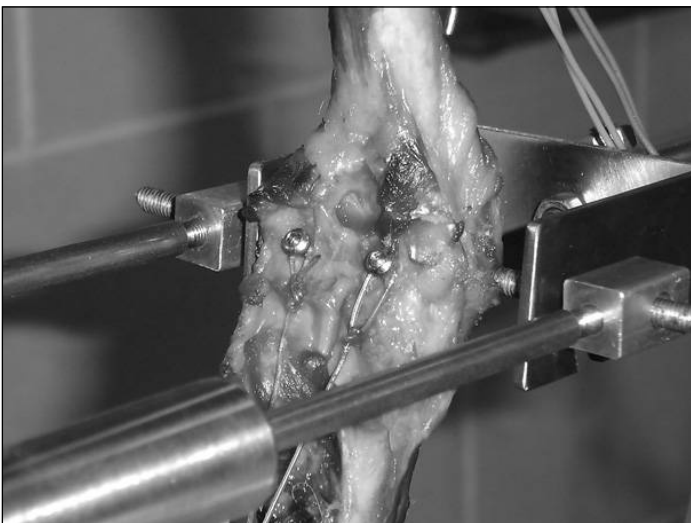

FIGURE 3. FORCE APPLICATION SYSTEM. A BAR TRANSMITS A TRANSVERSE FORCE ON THE CONDYLES IN ORDER TO SIMULATE THE MOVEMENT OF THE FEMUR RELATIVE TO THE TIBIA.

Specimen preparation with the TTA system

A longitudinal osteotomy was performed from the proximal cranial portion of the tibia, at the extensor sulcus level, to the distal area of the tibial crest, where a 3.5 mm orifice was made, as described in the tibial tuberosity advancement technique by Montavon *et al.* [12]. To perform the TTA, the tibial cranial fragment was progressively advanced, and a 9 mm box introduced and anchored in position with a 2 mm cortical screw. The plate was fixed with 2 mm cortical screws in its cranial portion and 2.7 mm screws in its caudal portion (FIG. 4).

FIGURE 4. POSITIONING OF THE DEVICE ON THE SPECIMEN TO BE TESTED, AND FIXATION WITH SCREWS.

Measuring systems

An orifice was drilled on the femoral condyles, and a 150 mm horizontal bar with M5 metric threaded was introduced to transmit a transverse force on the condyles in order to simulate the movement of the femur relative to the tibia (FIG. 4).

A force parallel to the tibial plateau was applied using 1 and 2 kg standard weights, and the values corresponding to the applied force and the displacement of the femur relative to the tibia for the three cases were recorded: full knee, CrCL-deficient knee and knee repaired with the TTA system. Due to the relative motion that exists between femur and tibia, the caudal femoral displacement on a fixed tibia is equivalent to the cranial displacement of the tibia on a fixed femur. The results shown in graphs and tables refer to the displacement of the tibia relative to the femur. Each type of experiment was performed three times in order to verify the repeatability of the response. The mean value of these three measurements was used for the elaboration of charts and tables. Force and displacement values were recorded with the help of a force sensor and two displacement sensors (FIG. 2). The force sensor was a tension load cell[2]. The displacement sensors used were inductive, Linear Variable Differential Transformer (LVDT)[3], with a sensitivity of $5 \cdot 10^{-4}$ mm. Their operation is based on the magnetic induction of an electric current whose intensity depends on the position of the core relative to the coil. The voltage signal of the generated electrical current is directly proportional to the distance travelled by the transducer core. The sensors, properly calibrated, provided a relationship between the voltage in volts and the displacement in millimetres. The displacement was calculated by adding the signals of both sensors and dividing the value by two.

The sensors were connected to a multiplexer[4] to treat and amplify the signal. A data acquisition card converted the analogue signal into a digital signal which was treated by a software designed using the *Laboratory Virtual Instrument Engineering Workbench* (LabView)[5] Management Program. This program was responsible for the reading management of all channels on the acquisition card, and for displaying and saving all the generated data in files.

Statistical analysis

TOne way analysis of variance, ANOVA, with eight specimens was used to compare changes in knee stiffness between intact knee, CrCL-deficient and TTA surgery. The confidence limits were 95%. It was obtained a P value < 0.001 and, therefore, the values depend on whether the knee was intact, CrCL-deficient or repaired with TTA. The absolute and relative values of stiffness were calculated based on the displacements. Normality of residuals was met by all values on the displacement table, and variability of residuals was similar in the three groups (assumption of homoscedasticity); there are no residuals outliers.

RESULTS AND DISCUSSION

The displacement values, as well as the absolute and relative knee stiffness values to the cranial displacement of the tibia relative to the femur were shown. The software was programmed to record 10 force and displacement data per second in each trial. The large number of data collected and the sensors' high sensitivity allowed highly accurate performance curves to be produced.

In the figure corresponding to specimen 2 (FIG. 5), the applied tangential force is related to the tibial displacement relative to the femur. In the graphs obtained from the 8 specimens, the relationship between force and displacement can be approximated to a straight line between 100 and 200 N.
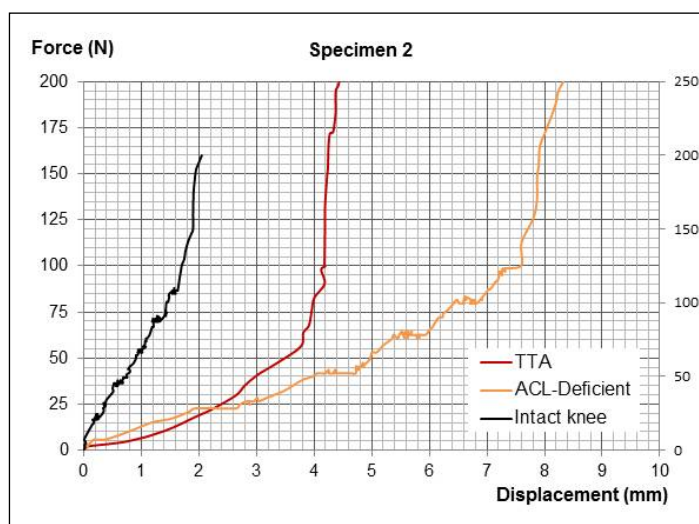

FIGURE 5. CRANIAL DISPLACEMENT OF THE TIBIA RELATIVE TO THE FEMUR (SPECIMEN 2).

The tables show the displacement and absolute and relative stiffness for a force value of 100 N (TABLES I, II and III). The absolute stiffness was obtained by dividing the 100 N force between the displacement in mm. Relative stiffness is the percentage of stiffness in the CrCL-deficient knee and the TTA repaired knee in relation to the intact knee. Mean values and their variation for a 95% confidence level (CL) were given at the end of each table.

The trend in all specimens is the increase of the displacement of the tibia relative to the femur after CrCL section. After TTA, displacement decreases but intact knee values were not recovered. Displacement was 1.70 ± 0.39 mm for the intact knee, 6.41 ± 0.627 mm for the CrCL-deficient knee, and 3.15 ± 0.59 mm for the TTA-repaired knee (TABLE I).

Knee stiffness to the displacement of the tibia relative to the femur decreased after CrCL section in the 8 specimens tested. After TTA, stiffness increases but the intact knee values were not recovered. Stiffness was 64.82 ± 26.08 N/mm for the intact knee, and 15.78 ± 1.79 N/mm for the CrCL-deficient knee. And for the

TTA repaired knee, it was 33.26 ± 7.85 N/mm (TABLE II).

Relative stiffness decreases after CrCL section. Relative stiffness increases to 57.88% after TTA, but fails to reach the 100% value of the intact knee (TABLE III).

Experimental *in-vitro* tests with 8 specimens are used to measure knee displacement and absolute and relative stiffness to cranial displacement of the tibia relative to the femur, and the values were compared among the three cases studied: intact knee, CrCL-deficient knee and TTA-device repaired knee. The most important limitations for this study were the great distance between the behaviour of the stifle joint in the specific *in-vitro* conditions of the study and the *in-vivo* stifle joint. Because of this, the analysis of the behaviour trends in displacement and stiffness from the data obtained in the tests was considered more important than the analysis of the absolute displacement values obtained when testing the three cases. It was worked with 8 *in-vitro* specimens. The data obtained from the results of the experimental trials were sufficient to meet the study objectives. Due to a lack of studies that directly apply a tangential force, it has not been possible to compare the results. Some studies attempt to reproduce the dog's gait by applying a force equivalent to 30% of the dog's weight [1, 23] or a force equal to 20% of the dog's weight in the axial direction of the tibia [10]. This study did not simulate the dog's gait. A horizontal force was applied to the femur, at the level of the condyle bones that simulated the tangential component of the knee. This was the component that caused the displacement of the tibia relative to the femur. The displacements were measured with LVDT, whose sensitivity was superior to other measuring systems such as the dial indicator [10] or the displacement measurement based on radiographs [1]. Due to a lack of studies that directly apply a tangential force, it has not been possible to compare the results.

The results showed that TTA stops the advance of the tibia relative to the femur. Other studies reach the same conclusion [1, 6, 8, 10]. Some of them measure the movement of the tibia relative to the femur [1]. However, there are no veterinary studies that quantify stifle stiffness to the cranial displacement of the tibia relative to the femur for the three cases.

The first objective of the study was to compare the stiffness of the intact knee, the CrCL -deficient knee and the TTA knee. The stiffness mean values were 64.82 N/mm for the intact knee, 15.78 N/mm for the CrCL-deficient knee and 33.26 N/mm for the TTA repaired knee.

The next objective was to know the loss of stiffness in the knee

TABLE II
STIFFNESS TO CRANIAL DISPLACEMENT OF THE TIBIA RELATIVE TO THE FEMUR (N/mm) FOR F = 100N, IN CADAVER KNEES (N = 8). ANOVA ANALYSIS

	Intact Knee	ACL-deficient	TTA
knee 1	71.43	16.95	35.71
knee 2	66.67	13.16	23.81
knee 3	43.48	15.87	30.30
knee 4	50.00	16.13	43.48
knee 5	125.00	17.54	45.45
knee 6	55.56	14.49	25.64
knee 7	47.62	13.89	29.41
knee 8	58.82	18.18	32.26
Mean	64.82	15.78	33.26
Standard deviation	26.08	1.79	7.85

P = 0.000; R-sq = 86.70%.

TABLE I
DISPLACEMENT OF THE TIBIA RELATIVE TO THE FEMUR (mm) FOR F = 100 N, IN CADAVER STIFLE JOINTS (N = 8). ANOVA ANALYSIS

	Intact Knee	ACL-deficient	TTA
knee 1	1.40	5.90	2.80
knee 2	1.50	7.60	4.20
knee 3	2.30	6.30	3.30
knee 4	2.00	6.20	2.30
knee 5	0.80	5.70	2.20
knee 6	1.80	6.90	3.90
knee 7	2.10	7.20	3.40
knee 8	1.70	5.50	3.10
Mean	1.70	6.41	3.15
Standard deviation	0.47	0.75	0.70

P = 0.000; R-sq = 90.37%.

TABLE III
RELATIVE STIFFNESS TO CRANIAL DISPLACEMENT OF THE TIBIA RELATIVE TO THE FEMUR (%) FOR F = 100N, IN CADAVER KNEES (N = 8). ANOVA ANALYSIS

	Intact Knee	ACL-deficient	TTA
knee 1	100.00	23.73	50.00
knee 2	100.00	19.74	35.71
knee 3	100.00	36.51	69.70
knee 4	100.00	32.26	86.96
knee 5	100.00	14.04	36.36
knee 6	100.00	26.09	46.15
knee 7	100.00	29.17	61.76
knee 8	100.00	30.91	54.84
Mean	100.00	28.34	57.88
Standard deviation	0.00	5.62	16.83

P = 0.000; R-sq = 90.57%.

after ACL excision compared to the intact knee, which was 76%.

The final objective was to obtain the stiffness increase of the knee after the implantation of a TTA device, which was found to be 210% compared to the CrCL-deficient knee.

CONCLUSIONS

Summarizing, as the applied tangential force increases, so does the tibial cranial displacement.; by sectioning the CrCL, displacement increased 3,8 times and the stiffness value decreased greatly (76%) compared to the intact knee; after the application of a TTA device, the healthy knee stiffness value was not reached, but the displacement was reduced by half and stiffness doubles compared to the CrCL-deficient knee. The TTA device does not stop cranial displacement of the tibia relative to the femur entirely, nor does it allow for recovery of the intact knee stiffness value; however, the stiffness value of the repaired knee doubled that of the CrCL-deficient knee.

BIBLIOGRAPHIC REFERENCES

[1] APELT, D; KOWALESKI, MP; BOUDRIEAU, RJ. Effect of tibial tuberosity advancement on cranial tibial subluxation in canine cranial cruciate-deficient stifle joints, an *in vitro* experimental study. **Vet. Surg.** 36:170-7. 2007

[2] ARNOCZKY, SP; MARSHALL, JL. Cruciate Ligaments of Canine Stifle - Anatomical and Functional-Analysis. **Am. J. Vet. Res.** 38:1807-14. 1977.

[3] BOUDRIEAU, RJ. Tibial Plateau Leveling Osteotomy or Tibial Tuberosity Advancement? **Vet. Surg.** 38: 1-22. 2009.

[4] BUTLER, JR; SYRCLE, JA; MCLAUGHLIN, RM; ELDER, SH. The effect of tibial tuberosity advancement and meniscal release on kinematics of the cranial cruciate ligament-deficient stifle during early, middle, and late stance. **Vet. Comp. Orthop. Traumatol**. 24:342-9. 2011.

[5] ELKINS, AD; PECHMAN, R; KEARNEY, MT. A retrospective study evaluating the degree of degenerative joint disease in the stifle joint of dogs following surgical repair of anterior cruciate ligament rupture. **J. Am. Anim. Hosp. Assoc.** 27:533-40. 1991.

[6] HOFFMANN, DE; KOWALESKI, MP; JOHNSON, KA; EVANS, RB; BOUDRIEAU, RJ. Ex Vivo Biomechanical Evaluation of the Canine Cranial Cruciate Ligament-Deficient Stifle with Varying Angles of Stifle Joint Flexion and Axial loads after Tibial Tuberosity Advancement. **Vet. Surg.** 40:311-20. 2011.

[7] KIM, SE; POZZI, A; BANKS, SA; CONRAD, B.P; LEWIS, DD. Effect of Tibial Tuberosity Advancement on Femorotibial Contact Mechanics and Stifle Kinematics. **Vet. Surg.** 38:33-9. 2009.

[8] KIPFER, NM; TEPIC, S; DAMUR, DM; GUERRERO, I; HAESSIG, M; MONTAVON, PM. Effect of tibial tuberosity advancement on femorotibial shear in cranial cruciate-deficient stifles an *in vitro* study. **Vet. Comp. Orthop. Traumatol.** 21:385-90. 2008.

[9] LAFAVER, S; MILLER, NA; STUBBS, WP; TAYLOR, RA; BOUDRIEAU, RJ. Tibial tuberosity advancement for stabilization of the canine cranial cruciate ligament-deficient stifle joint, Surgical technique, early results, and complications in 101 dogs. **Vet. Surg.** 36:573-86. 2007.

[10] MILLER, JM; SHIRES, PK; LANZ, OI; MARTIN, RA; GRANT, JW. Effect of 9 mm tibial tuberosity advancement on cranial tibial translation in the canine cranial cruciate ligament-deficient stifle. **Vet. Surg.** 36:335-40. 2007.

[11] MONTAVON, PM; DAMUR, DM; TEPIC, S. Advancement of the tibial tuberosity for the treatment of cranial cruciate deficient canine stifle. **Proceedings 1st World Orthopaedic Veterinary Congress**. Munich, 09/5-8, Germany. Pp 152. 2002.

[12] MONTAVON, PM; DAMUR, DM; TEPIC, S. Tibial tuberosity advancement (TTA) for the treatment of cranial cruciate disease in dogs, evidences, technique and initial clinical results. **Proceedings 12th ESVOT Congress**. Munich, 09/10-12, Germany. Pp 254-255. 2004.

[13] NESS, MG; ABERCROMBY, RH; MAY, C; TURNER, B; CARMICHAEL, S. A survey of orthopaedic conditions in small animal veterinary practice in Britain. **Vet. Comp. Orthop. Traumatol.** 9:43-52. 1996.

[14] SCHWARZ, PD. Tibial plateau leveling osteotomy (TPLO), A prospective clinical comparative study. **Proceedings 9th Annual ACVS Symposium**. San Francisco, 09/30-10/03, USA. Pp 379-380. 1999.

[15] SHAHAR, R; BANKS-SILLS, L. Biomechanical analysis of the canine hind limb, Calculation of forces during three-legged stance. **Vet. J.** 163, 240-50. 2002.

[16] SLOCUM, B; SLOCUM, TD. Tibial Plateau Leveling Osteotomy for Repair of Cranial Cruciate Ligament Rupture in the Canine. **Vet. Clin. North. Am. Anim. Pract.** 23:777-95. 1993.

[17] STEIN, S; SCHMOEKEL, H. Short-term and eight to 12 months results of a tibial tuberosity advancement as treatment of canine cranial cruciate ligament damage. **J. Small. Anim. Pract.** 49:398-404. 2008.

[18] TEPIC, S; DAMUR, DM; MONTAVON, PM. Biomechanics of the stifle joint. **Proceedings 1st World Orthopaedic Veterinary Congress**. Munich, 09/5-8, Germany. Pp 189-190. 2002.

[19] TEPIC, S; MONTAVON, PM. Is cranial tibial advancement relevant in the cruciate deficient stifle? **Proceedings 12th ESVOT Congress.** Munich, 09/10-12, Germany. Pp 132-133. 2004.

[20] TEPIC, T. Cranial tibial tuberosity advancement for the cruciate deficient stifle. **Proceedings 2nd World Veterinary Orthopaedic Congress.** Keystone, 02/25-03/4, USA. Pp 44-45. 2006.

[21] VASSEUR, PB; BERRY, CR. Progression of stifle osteoarthrosis following reconstruction of the cranial cruciate ligament in 21 dogs. **J. Am. Anim. Hosp. Assoc.** 28:129-36. 1992.

[22] VEZZONI, A. TTA (tibial tuberosity advancement) in the treatment of cranial cruciate ligament injuries. **Proceedings North American Veterinary Conference**. Orlando, 01/7-11, USA. Pp 949-952. 2006.

[23] WARZEE, CC; DEJARDIN, LM; ARNOCZKY, SP; PERRY, RL. Effect of tibial plateau leveling on cranial and caudal tibial thrusts in canine cranial cruciate-deficient stifles, an *in vitro* experimental study. **Vet. Surg.** 30:278-86. 2001.

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