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EXPERIMENTAL LAB RESEARCH

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Evaluation of the accuracy and intra- and interobserver reliability of three manual laxity tests for canine cranial cruciate ligament rupture—An ex vivo kinetic and kinematic study

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Abstract

Objectives: To investigate the accuracy and intra- and interobserver reliability of the cranial drawer test (CD), tibial compression test (TCT), and the new tibial pivot compression test (TPCT) in an experimental setting resembling acute cranial cruciate ligament rupture (CCLR) and to elucidate the ability to subjectively estimate cranial tibial translation (CTT) during testing.

Study design: Experimental ex vivo study.

Sample population: Ten cadaveric hindlimbs of large dogs.

Methods: Kinetic and 3D-kinematic data was collected while three observers performed the tests on each specimen with intact (INTACT) and transected cranial cruciate ligament (CCLD) and compared using three-way repeated-measures ANOVA. Subjectively estimated CTT (SCTT), obtained during a separate round of testing, was compared to kinematic data by Pearson correlation.

Results: CTT was significantly higher for CCLD than for INTACT for all tests, resulting in 100% sensitivity and specificity. TPCT induced the highest CTT and internal rotation. Intra- and interobserver agreement of translation was excellent. For rotation and kinetics, agreement was more variable. SCTT strongly correlated with the objectively measured values.

Conclusion: The CD, TCT and the new TPCT were all accurate and reliable. The high translations and rotations during TPCT are promising, encouraging further development of this test. SCTT was reliable in our experimental setting.

Clinical significance: Veterinary manual laxity tests are accurate and reliable in acute CCLR. The TPCT might have potential for the assessment of subtle and rotational canine stifle instabilities. The high reliability of SCTT implies

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that grading schemes for stifle laxity, similar to human medicine, could be developed.

1 | INTRODUCTION

Cranial cruciate ligament rupture (CCLR) is a common disease in canine orthopedics.[1,2] Most CCLR cases are treated surgically, although subclassifying CCLR based on clinical presentation may help defining more specific treatment recommendations.[3,4] CCLR is diagnosed primarily by physical examination, often by manual laxity tests (MLT) such as the cranial drawer test (CD) and the tibial compression test (TCT).[5,6] Excessive cranial tibial translation (CTT) during testing suggests CCLR.[6] Although the TCT and CD are routinely used, they were never validated using objective kinetic or kinematic measures.[7] Furthermore, a recent study reported a surprisingly low accuracy for the CD and TCT.[7] This could be caused by interobserver variability of the testing maneuver, as shown for some MLTs in humans.[8,9] To reduce interobserver variability and increase accuracy of MLTs for human anterior cruciate ligament rupture (ACLR), standardized testing maneuvers have been successfully established.[10] With the same purpose, quantitative measurement methods for human MLTs have been developed.[9,11–14] So far, there are no veterinary studies investigating strategies to standardize the CD or TCT or to establish methods for subjective and objective quantification of CTT during MLTs.

In addition to limiting CTT, the cranial cruciate ligament (CCL) restrains internal tibial rotation.[15] Rotational instability after CCLR has become increasingly recognized in dogs.[16–19] So far, there is no veterinary test to assess for this type of instability, although it would be valuable to better characterize stifle instability and to identify dogs that might be prone to complications after surgical treatment of CCLR.[16,19] In humans, rotational instability in addition to anterior laxity is a well-known problem after ACLR and is evaluated by the pivot shift test.[20–23] For this test, the patient lies in dorsal recumbency, legs extended. The examiner picks up the affected leg at the ankle and applies internal rotation with one hand. The other hand is placed proximolateral on the tibia and applies a valgus stress. Then, the knee is slowly flexed. In ACLR, a sudden reduction of the anteriorly subluxated lateral tibial plateau can be palpated.[21] Based on the pivot shift test, the authors developed a new test named tibial pivot compression test (TPCT) to detect rotational and craniocaudal instability in canine patients. The TPCT can be performed with the dog in dorsal or lateral recumbency and consists of a standard TCT combined with a rotational and a valgus stress.

Considering all the above, this study had four objectives. First, we wanted to describe and compare the kinetics and kinematics of the canine stifle joint during the CD, TCT and the new TPCT when performed by three different observers in an experimental set up including intact and transected CCLs. Second, we used kinematic and kinetic data to assess the intra- and interobserver reliability of the three MLTs. Third, to assess the accuracy of subjective quantification of CCT, we compared the subjectively estimated CTT to the objective CTT value measured during the tests. Our fourth objective was to evaluate the new TPCT for assessing rotational instability. Based on our clinical experience and preliminary data, we hypothesized (1) that the TPCT would elicit more CTT and internal tibial rotation than the other tests, (2) that the intraobserver reliability of kinetics and kinematics of the tests is better than the interobserver reliability, and (3) that all tests are accurate at detecting CTT but unreliable for quantifying it.

2 | MATERIALS AND METHODS

2.1 | Specimen preparation

Ten pelvic limbs of skeletally mature dogs weighing >22 kg were collected. Left or right limbs were randomly selected. All dogs were euthanized for reasons unrelated to this study and donated for research by their owners. To exclude stifle pathologies, orthogonal radiographs of the joints were obtained and stifle arthroscopy was performed, including inspection and probing of the CCL, the caudal cruciate ligament and the menisci. The limbs were disarticulated at the coxofemoral joint and the proximal half of the femur was freed from soft tissues. The proximal part of the femur was osteotomized using an oscillating saw and the remainder of the femoral diaphysis was potted centrally in a 3D-printed cylinder using beracryl-monomer (SCS-Beracryl D-28 monomer; Swiss-Composite, Fraubrunnen, Switzerland). Fur was clipped from the femur to distal to the stifle joint. After preparation, the specimens were stored at −20°C and thawed to room temperature 24 h before testing.

2.2 | Setup and testing protocol

The specimens were mounted on a custom-made 3D-printed jig (3DGence ONE, 3DGence, Przyszowice, Polska) reinforced with beracryl-monomer (Swiss-Composite) and clamped to a table using carpenter clamps (Figure 1). To maintain the stifle joint at a standing angle (135°) throughout testing, limb position was set by an adjustable support bar and rechecked before each test with a goniometer. A load cell (S-Type Load Cell, range ±10 kgF, Omega Engineering, Manchester, UK) was inserted between the jig and the specimen to register axial load applied to the femur during the TCT and the TPCT (Figure 2). For the assessment of kinetics during CD, a subminiature load cell (Subminiature Compression Load Cell, ±10 kgF, Omega Engineering, Manchester, UK), fixed to the observer's thumb using a self-adherent wrap, registered the compressive force applied to the fibular head (Figure 2).

To allow tracking of 3D kinematics, reflective markers forming a custom-made coordinate system were attached to the femur and tibia of each specimen (Figure 1). The coordinate systems consisted of three 2.5 mm pins, a 3D-printed central connecting part, and five spherical reflective markers, a standard set up for motion capture analysis. The markers were glued to the pins and central part in a pattern unique for femur and tibia respectively to allow distinction of the bones by the

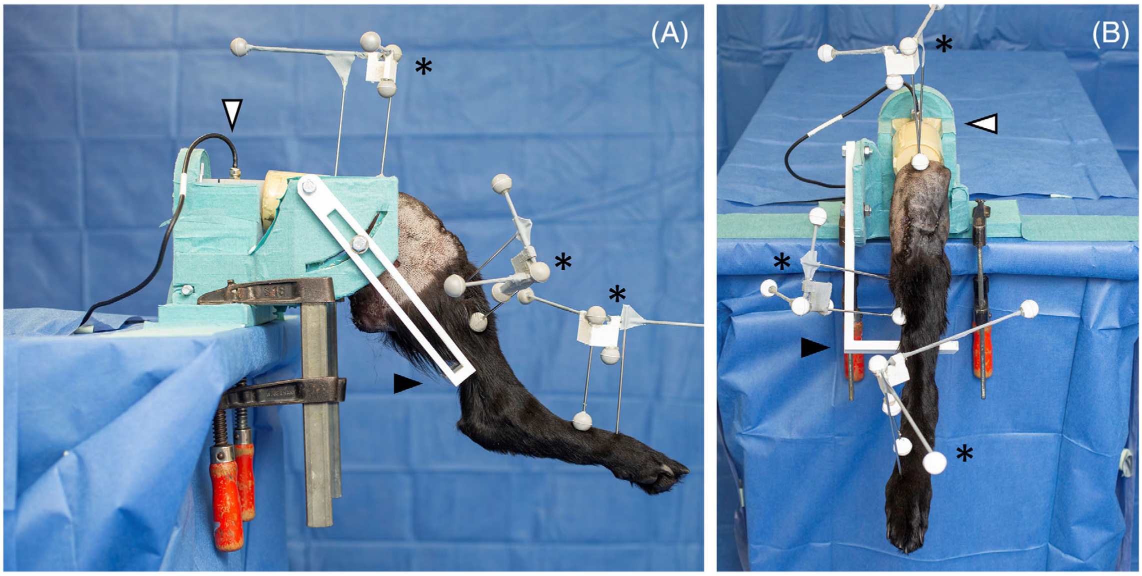

FIGURE 1 Testing set up. Medial (A) and cranial (B) view showing the set up with the femoral load cell (▷) and the mounted specimen with coordinate systems for tracking (*) held in position by an adjustable support bar (►).

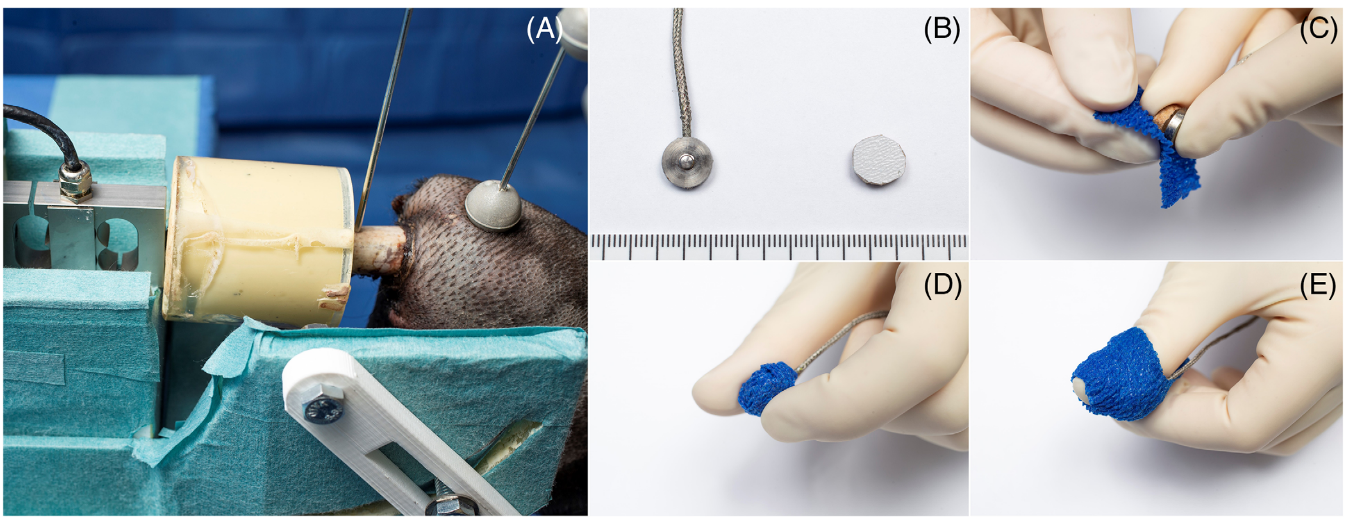

FIGURE 2 Load cells. (A) Medial close-up of the S-type load cell inserted between the specimen and the jig. (B–E) Image series showing the fixation of the subminiature load cell used during the cranial drawer test.

tracking software (Qualisys Track Manager, Qualisys, Gothenburg, Sweden) (Figure 1).

Ten motion capture cameras (Qualisys, Gothenburg, Sweden) sampling at 300 Hz collected kinematic data by tracking the motions of the reflective markers. Data was recorded and edited by QTM software (Qualisys Track Manager, Qualisys).

The testing protocol included three rounds of testing (Figure 3). In all rounds, all tests were performed by three observers with different levels of experience (board-certified surgeon, [observer 1], resident [observer 2], doctoral student [observer 3]) on each specimen. In the first round (INTACT), the CCL was intact in all specimens. In round 2, the accuracy of the tests was assessed and CTT was subjectively estimated to later compare it to the objective values obtained by kinematic assessment. For this round, the CCL of five randomly selected limbs was transected arthroscopically by a board-certified surgeon. The remaining limbs underwent sham-arthroscopy. The arthroscopy incisions were closed routinely by single interrupted sutures. The observers were unaware of the state of the CCL to allow blind assessment of each specimen. During each test, each limb was assessed qualitatively (CCL intact/CCL transected) as well as quantitatively (estimation of CTT in mm) by palpation only by all observers. For testing in round 3 (CCLD), the CCL was transected in all limbs and testing was repeated as described for round 1. The order of tests, specimens and observers was chosen randomly in each round by one of the investigators. For the assessment of intraobserver reliability, the tests were repeated three times in three randomly selected specimen for INTACT and CCLD.

2.3 | Manual laxity tests

The three MLTs CD, TCT and TPCT were evaluated. All tests were performed with the observer standing lateral to the specimen. For the CD, the femur was stabilized with one hand, while the thumb of the other hand was placed behind the fibular head and the index finger on the tibial tuberosity. After applying a caudally directed force to the tibia to reduce the joint, the observer pushed the tibia cranially to detect excessive cranial tibial motion in the sagittal plane. The pressure applied was measured by a subminiature load cell secured to the observer's thumb, as described before.

For the TCT, the observer's hands were placed on the tibia, as described by Henderson and Milton.[5] Instead of stabilizing the femur with one hand, only the index finger of this hand was placed on the tibial tuberosity to detect excessive CTT without interfering with the femoral load cell measurements. Axial tibial compression was applied by flexing the tarsal joint with the stifle and tarsus aligned in the sagittal plane. The tarsus was held in a neutral position during testing.

The TPCT was performed similarly to a standard TCT. However, before initiating tibial compression, the

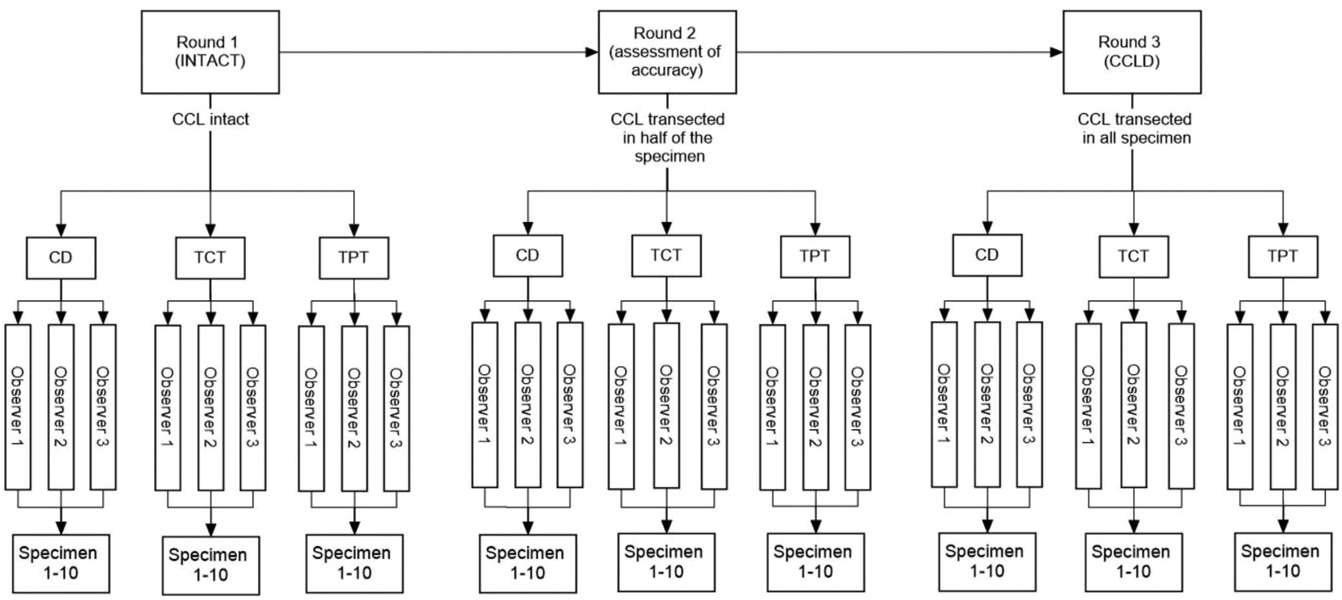

FIGURE 3 Testing procedure. Testing was conducted in three rounds. In each round of testing each observer performed each test on each specimen. For round 1, the CCL was intact in all specimens. In round 2, the CCL was transected in five randomly selected specimen to allow blind qualitative and quantitative assessment of tibial translation. In round 3, the CCL was transected in all specimens. CCL cranial cruciate ligament.

tarsus was brought in external rotation until resistance was felt and a valgus stress was applied. Then tibial compression was established, and external rotation was released, allowing the tibia to internally rotate and eventually subluxate (Figure 4) (Video S1).

2.4 | Data processing

Directly after testing, computed tomography (CT) scans of all specimens, including the associated coordinate systems attached in the exact same position as during testing, were obtained. From the CT scans, femur and tibia and their respective coordinate system were segmented using 3D Slicer software (version 4.10.1, stable release[24]) to create 3D models. The Geomagic WRAP software (Geomagic Inc., Research Triangle Park, North Carolina, USA) was then used to apply an anatomical coordinate system matching the 3D models as described in previous studies (Figure 5).[25,26] From these models and the motion capture data, peak tibial translation (mm), peak tibial axial internal or external rotation (degree) and peak stifle joint flexion (degree) were calculated using a custom-written program in MATLAB (The Mathworks Inc., Natick, Massachusetts, USA). Peak tibial axial rotation in degree is described as a negative (internal rotation) or positive value (external rotation) in relation to the starting point of the test. The kinetic data obtained by the

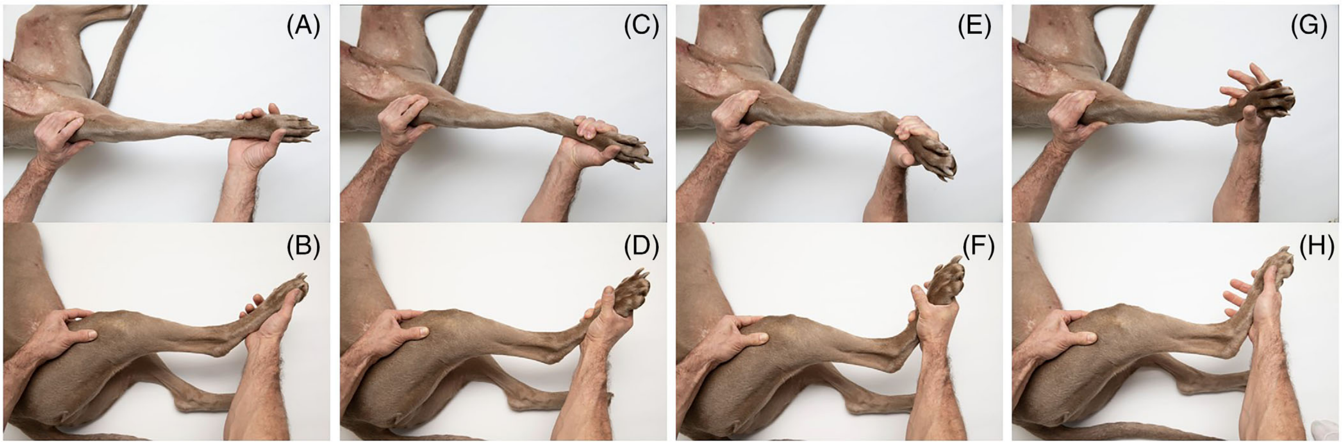

FIGURE 4 Testing maneuver of the tibial pivot compression test. Top row showing the cranial and bottom row showing the lateral view. (A, B) Starting position. (C,D) External rotation and valgus stress are applied. (E, F) Tibial compression is established. (G, H) Release of rotation and eventual subluxation in cranial cruciate ligament – deficient stifle.

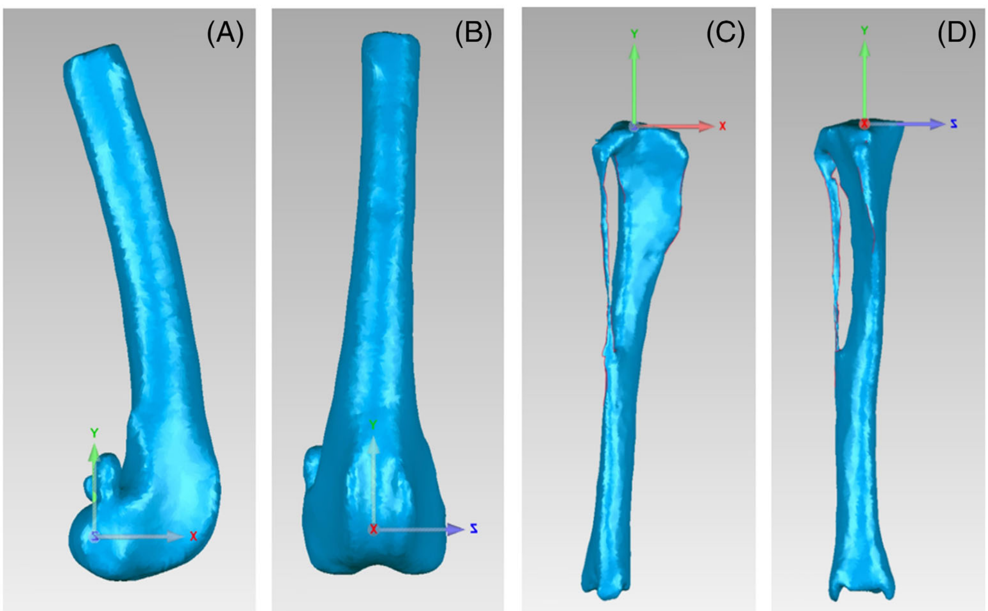

FIGURE 5 Three-dimensional models of femur and tibia created from computed tomography scans. Mediolateral (A) and craniocaudal (B) view of the femur and lateromedial (C) and craniocaudal (D) view of the tibia. A previously described anatomical coordinate system matching the one used during testing was applied to the models.[25,26]

load cells as a force in N was filtered for analysis using first order Butterworth filter with a cut off frequency of 1 Hz and a sampling rate of 200 Hz by MATLAB (The Mathworks Inc.).

2.5 | Statistical analysis

The data were analyzed using SPSS (version 25.0 IBM Corp., Armonk, New York, USA). Descriptive values are reported as mean ± standard deviation (SD). Flexion data were compared between INTACT and CCLD using a paired *t*-test. These data were not included in further analysis, as stifle joint angle was a controlled variable throughout testing (target angle = 135°). A three-way repeated-measures ANOVA was conducted to investigate peak tibial translation and peak tibial rotation differences. Within-subject factors were CCL state (INTACT, CCLD), test (CD, TCT, TPCT) and observer (observer 1, 2, 3). External rotation at the start of the TPCT was not included in the ANOVA analysis, as it was elicited by the observer and not a result of the test. External rotation data was compared between INTACT and CCLD using a paired *t*-test. To account for the two different sensors assessing kinetics during testing, the kinetics of the CD were evaluated separately from the kinetics of the TCT and TPCT. A two-way repeated-measures ANOVA was conducted for CD. Within-subject factors were CCL state (INTACT, CCLD) and observer (observers 1, 2, 3). TCT and TPCT were directly compared using a three-way repeated-measures ANOVA identical to the analysis for translation and rotation. If two- or three-way models revealed interactions between within–subject factors, the analysis was split up in two- or one-way models, respectively, until no more interactions were present.

Sphericity of the data was evaluated using the Mauchly test, and Greenhouse–Geisser correction was used as indicated. In the case of statistically significant ANOVA differences ($p \leq .05$), post hoc testing was used for pairwise comparison, using a statistical significance set by the Bonferroni correction, which controls type I error inflation. To evaluate inter- and intraobserver reliability of kinematic and kinetic data, intraclass correlation coefficients (ICC) and corresponding 95% confidence intervals were calculated using a two-way mixed, absolute agreement model.[27] An ICC of <0.5 was classified as poor agreement, 0.5–0.74 as moderate agreement, 0.75–0.89 as good agreement, and >0.9 as excellent agreement.[27] Subjective quantitative assessment of CTT was compared to the actual translation values measured by the motion capture system using Pearson's correlation. Median absolute difference between subjective and objective values was calculated.

3 | RESULTS

Six right and four left limbs were collected. Mean bodyweight ± SD of the dogs was 31.5 ± 5.4 kg and mean age ± SD was 8.6 ± 3.1 years. Mean deviation from the target stifle flexion angle of 135° during testing was −1.86° ± 5.03°. There was significantly more variation in flexion angle for CCLD than for INTACT ($p = .001$).

3.1 | Translation

CTT was significantly higher for CCLD than for INTACT ($p < .001$) (Table 1). No caudal tibial translation occurred during testing. The highest CTT's were elicited during TPCT for both INTACT and CCLD (Table 1). The discrepancy between the three tests was not significant for INTACT ($p = .30$); however, for CCLD, the recorded translation was significantly higher for TPCT than for CD ($p = .003$). There was no significant difference between TPCT and TCT for CCLD ($p = .97$). For INTACT, the most experienced observer 1 elicited significantly more translation during CD than the other two observers (observer 1 – observer 2: $p = .014$, observer 1 – observer 3: $p = .018$). There was no significant difference in CTT for the other observers or tests. Inter- as well as intraobserver agreement of CTT were excellent (Tables 4 and 5).

3.2 | Rotation

There was significantly more rotation for CCLD than for INTACT ($p = .03$) (Table 2). Ultimately, all three tests resulted in net internal rotation. The highest values of internal rotation were elicited by TPCT (mean CD INTACT + CCLD: 5.95° ± 7.36°, mean TCT INTACT + CCLD: −6.39° ± 6.41°, mean TPCT INTACT + CCLD: −9.13° ± 9.20°). The differences between the three tests and between observers were not significant (tests: $p = .141$, Observers: $p = .074$).

Mean peak external tibial rotation applied at the start of TPCT was consistent during testing ($p = .22$; mean rotation INTACT: 12.75 ° ± 4.06°, mean rotation CCLD: 14.14° ± 5.7°). Interobserver agreement of tibial rotation during CD was only moderate (ICC = 0.54), while it was good during TPCT (ICC = 0.76) and TCT (ICC = 0.87) (Table 4). Intraobserver agreement was good to excellent except for CD and TCT performed by observer 3 (ICC CD = 0.45, ICC TCT = 0.58) (Table 5).

TABLE 1 Cranial tibial translation[a] during manual laxity tests.

	Observer 1	Observer 2	Observer 3	Mean
Translation in mm				
CD				
INTACT	2.99 (1.64)	0.55 (1.36)	1.4 (1.11)	1.65 (0.29)
CCLD	12.43 (3.08)	10.15 (2.47)	11.36 (1.89)	11.31 (0.58)
Difference[b]	9.43	9.59	9.96	
TCT				
INTACT	1.33 (1.15)	1.29 (0.97)	1.03 (0.76)	1.22 (0.2)
CCLD	12.6 (3.69)	11.54 (3.04)	11.6 (3.13)	11.91 (0.92)
Difference[b]	11.27	10.25	10.57	
TPCT				
INTACT	1.94 (1.27)	1.68 (0.94)	1.94 (1.25)	1.86 (0.3)
CCLD	14.15 (3.57)	12.65 (2.12)	13.61 (4.41)	13.47 (0.94)
Difference[b]	12.21	10.96	11.67	

Abbreviations: CCLD, cranial cruciate ligament deficient; CD, cranial drawer test; INTACT, intact cranial cruciate ligament; TCT, tibial compression test; TPCT, tibial pivot compression test.

[a]Shown as mean (standard deviation).

[b]Differences are the calculated mean of subtraction of INTACT values from CCLD values for each specimen per observer.

3.3 | Kinetics

The force applied during CD was significantly higher for INTACT than for CCLD ($p < .001$). During CD, the amount of force employed corresponded to the experience level of the observer: the more experience, the higher the applied force (Table 3).

For INTACT, the forces applied were significantly higher during TPCT than during TCT ($p = .021$). During INTACT, the more experienced observers 1 and 2 applied higher forces. However, this finding only reached significance between observers 2 and 3 ($p = .005$). For CCLD, there was no significant difference between the kinetics of the two tests except for the least experienced observer 3, who applied more force during TPCT than during TCT (observer 1: $p = .147$, observer 2: $p = .248$, observer 3: $p < .001$). Overall, it was found that during all tests, the forces applied during INTACT were higher than during CCLD (CD: $p < .001$, TCT: $p < .001$, TPCT: $p = .017$). Direct comparison of TCT and TPCT revealed that overall higher forces were applied during TPCT ($p < .001$).

Interobserver agreement of kinetics was poor for CD (ICC = 0.44), moderate for TPCT (ICC = 0.51), and good for TCT (0.82). Intraobserver agreement for CD was moderate (mean ICC = 0.69) while it was good for TCT and TPCT (mean ICC TCT = 0.87, mean ICC TPCT = 0.78). The best intraobserver agreement of kinetics was demonstrated by the most experienced observer 1 (mean ICC = 0.92), followed by the least experienced observer 3 (mean ICC = 0.89) (Tables 4 and 5).

3.4 | Subjective qualitative and quantitative assessment

The CTT for INTACT ranged between 0.1 and 5 mm and between 7 and 18 mm for CCLD. The three observers' subjective qualitative assessment of CTT revealed a sensitivity and specificity of 100% for all tests and all observers. Comparison of subjective quantitative assessment values estimated by the three observers to the objective kinematic values revealed a strong correlation with a correlation coefficient of 0.895 (Figure 6). Median absolute difference between subjective and objective values was 1.31 mm.

4 | DISCUSSION

Our study describes the kinematics and kinetics of MLTs for canine CCLR. All evaluated tests elicit a significantly higher CTT in CCL-deficient limbs than in those with intact CCL. The increase in internal tibial rotation and the reduced force required to achieve translation in the CCLD group reflect the compromised stifle joint stability after CCLR. Interobserver agreement for CTT was excellent for all tests, while there was more interobserver variability for rotation and kinetics. For example, the force applied when performing CD was highly variable, although the elicited CTT was very consistent. This result may be due to our model mimicking acute CCLR. In these hyperlax stifles, only minimal force is required to

TABLE 2 Rotation[a] during manual laxity tests.

	Observer 1	Observer 2	Observer 3	Mean
Rotation in degree				
CD				
INTACT	−5.8 (9.94)	−3.52 (7.93)	−2.38 (3.76)	−3.9 (1.74)
CCLD	−9.8 (10.67)	−8.16 (3.62)	−6.03 (3.35)	−7.97 (1.89)
Difference[b]	−4.0	−4.64	−3.65	
TCT				
INTACT	−4.36 (4.9)	−0.36 (3.79)	−2.3 (3.5)	−2.35 (2.0)
CCLD	−11.48 (4.14)	−9.19 (7.04)	−10.63 (5.53)	10.43 (1.16)
Difference[b]	−7.12	−8.83	−8.33	
TPCT				
External rotation				
INTACT	10.56 (3.38)	11.72 (3.58)	15.88 (3.49)	12.72 (4.09)
CCLD	11.64 (4.58)	13.72 (5.16)	18.97 (6.44)	14.78 (6.12)
Difference[b]	1.09	2.01	3.09	
Internal rotation				
INTACT	−11.11 (13.5)	−8.05 (14.62)	−1.22 (8.23)	−6.79 (5.06)
CCLD	−10.81 (5.6)	−10.4 (6.98)	−13.17 (7.47)	−11.46 (1.49)
Difference[b]	0.3	−2.35	−11.95	

Abbreviations: CCLD, cranial cruciate ligament deficient; CD, cranial drawer test; INTACT, intact cranial cruciate ligament; TCT, tibial compression test; TPCT, tibial pivot compression test.
[a]Shown as mean (standard deviation).
[b]Differences are the calculated mean of subtraction of INTACT values from CCLD values for each specimen per observer.

elicit CTT, which likely resulted in our observers using variable force but still achieving the same CTT. Another possible source of variation is the positioning of the load cell on the observer's thumb.

The highest tibial translation values were elicited by the TPCT, although this finding was only significant when compared to the CD. This finding confirms part of our first hypothesis. The TPCT was developed based on the human pivot shift test, adding external rotation and valgus stress to the TCT to make rotational instability better palpable. The external moment applied before establishing tibial compression displaces the lateral tibial condyle caudally, resulting in greater translation and rotation when the tibia subluxates. This effect of the external rotation may be particularly important in chronic CCLR, where the joint is often subluxated when starting the test. Possibly, the TPCT is a more sensitive test to detect subtle instability, as the tibial translation magnitude may influence the ability of the observer to feel tibial motion. The TPCT also elicited the highest internal rotation among all tests; however, this finding did not reach significance. A study with a higher number of specimens would be necessary to confirm the second part of our first hypothesis. Still, this finding seems promising and might make the TPCT a potential candidate to detect rotational instability. We found a high variability in rotation during TPCT in INTACT when compared to CCLD. This reflects our clinical experience and is most likely due to individual variability in screw home mechanism due to different articular surface geometry and soft tissue envelope.

The translation and rotation values reported here are slightly higher than the values reported in an in vivo study during walking.[28] This can be explained by different study populations, as the dogs in the in vivo study had naturally occurring chronic-degenerative CCLR, while our model mimics hyperlax stifles seen with acute CCLR. Interestingly, two in vitro studies found higher translation and rotation values in canine stifles with CCLR than reported here.[29,30] A possible reason is their specimen preparation as the specimen was stripped of most soft tissue surrounding the stifle joint, removing passive restraints of translation and rotation. Nevertheless, the compatibility of our results with these studies, especially the in vivo experiment during walking, suggests that the MLTs used to diagnose CCLR reproduce kinematics similar to weightbearing.

TABLE 3 Kinetic measurements[a] during manual laxity tests.

	Observer 1	Observer 2	Observer 3	Mean
Compressive force in N				
CD				
INTACT	3.5 (0.61)	2.4 (0.62)	1.76 (0.26)	2.6 (0.9)
CCLD	2.57 (0.37)	1.62 (0.23)	1.13 (0.34)	1.77 (0.68)
Difference[b]	−0.98	−0.77	−0.64	
Axial femoral force in N				
TCT				
INTACT	8.25 (3.67)	10.84 (4.4)	7.15 (3.1)	8.75 (3.96)
CCLD	5.1 (2.32)	5.42 (1.65)	3.49 (1.26)	4.67 (1.95)
Difference[b]	−3.15	−5.42	−3.66	
TPCT				
INTACT	9.3 (3.5)	12.2 (4.74)	10.76 (2.82)	10.76 (3.84)
CCLD	6.58 (3.63)	7.06 (4.1)	10.91 (2.0)	8.18 (3.82)
Difference[b]	−2.72	−5.15	0.15	

Abbreviations: CCLD, cranial cruciate ligament deficient; CD, cranial drawer test; INTACT, intact cranial cruciate ligament; TCT, tibial compression test; TPCT, tibial pivot compression test.
[a]Shown as mean (standard deviation).
[b]Differences are the calculated mean of subtraction of INTACT values from CCLD values for each specimen per observer.

While the CD is most frequently used in daily practice, its mean interobserver agreement for translation, rotation and kinetics was only moderate. Both TCT and TPCT had better interobserver agreement, with TCT being most consistent. This possibly makes those tests more reliable in the clinical setting. Despite a more standardized testing maneuver, interobserver agreement of TPCT was not significantly different from the other tests. This could be explained by the higher complexity and unfamiliarity of the testing maneuver. Intraobserver agreement was excellent for translation but only moderate to good for rotation and kinetics. Agreement was best for TCT and the most experienced observer, indicating that practicing MLTs helps improve their reliability. The second hypothesis can therefore be accepted as intraobserver agreement was moderate to excellent, while interobserver agreement was only moderate to good. The good intra- and interobserver agreement of the TCT shows that the slightly modified testing maneuver used to avoid interference with the femoral load cell did not impact consistency. However, we cannot exclude a slight alteration of the values due to this different testing maneuver.

Qualitative subjective assessment of stifle joint stability revealed a sensitivity and specificity of 100% for all tests and all observers, which is in line with the first part of our third hypothesis. This result should be interpreted carefully because our model using CCL transection mimics hyperlax stifles, which present with instability that is easily detected by MLTs.[4,6] Other available

TABLE 4 Interobserver agreement of kinetics and kinematics.

	ICC	95% Confidence interval	
		Lower	Upper
Translation			
CD	0.95	0.86	0.98
TCT	0.98	0.95	0.99
TPCT	0.98	0.95	0.99
Rotation			
CD	0.54	0.06	0.83
TCT	0.87	0.69	0.95
TPCT	0.76	0.50	0.89
Kinetics			
CD	0.44	0.06	0.76
TCT	0.82	0.57	0.93
TPCT	0.51	0.04	0.78

Note: Agreement is shown as intraclass correlation coefficient (ICC) and 95% confidence interval. ICC >0.9 = excellent, ICC >0.75 = good, ICC >0.5 = moderate, ICC <0.5 = poor agreement.[27]
Abbreviations: CD, cranial drawer test; ICC, intraclass correlation coefficient; TCT, tibial compression test; TPCT, tibial pivot compression test.

studies investigating the accuracy of CD and TCT found less favorable results.[7,31] Might et al. reported a sensitivity of 97% and a specificity of 82% for the CD for the classification of intact limbs and limbs with CCLR, caudal

TABLE 5 Intraobserver agreement of kinetics and kinematics.

	ICC	95% Confidence interval Lower	Upper
Translation			
CD			
O1	0.94	0.75	0.99
O2	0.96	0.85	0.99
O3	0.94	0.77	0.99
TCT			
O1	0.98	0.916	1.0
O2	0.99	0.966	1.0
O3	0.93	0.715	0.99
TPCT			
O1	0.95	0.805	0.99
O2	0.97	0.866	1.0
O3	0.97	0.883	1.0
Rotation			
CD			
O1	0.84	0.36	0.98
O2	0.92	0.64	0.98
O3	0.45	0.23	0.84
TCT			
O1	0.92	0.652	0.988
O2	0.92	0.63	0.98
O3	0.58	0.43	0.94
TPCT			
O1	0.77	0.16	0.97
O2	0. 77	0.01	0.97
O3	0.91	0.64	0.99
Kinetics			
CD			
O1	0.97	0.88	1.0
O2	0.23	0.12	0.88
O3	0.88	0.55	0.98
TCT			
O1	0.96	0.85	1.0
O2	0.92	0.99	0.96
O3	0.99	0.95	1.0
TPCT			
O1	0.91	0.63	0.99
O2	0.67	−0.11	0.95
O3	0.79	0.07	0.97

Note: Agreement is shown as intraclass correlation coefficient (ICC) and 95% confidence interval. ICC >0.9 = excellent, ICC >0.75 = good, ICC >0.5 = moderate, ICC <0.5 = poor agreement.[27]
Abbreviations: CD, cranial drawer test, ICC, intraclass correlation coefficient; TCT, tibial compression test; TPCT, tibial pivot compression test.

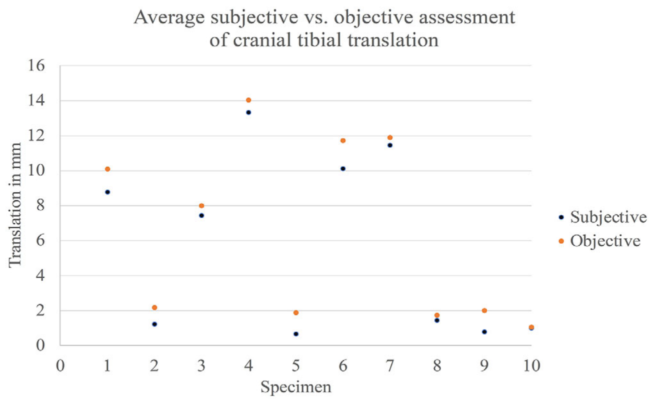

FIGURE 6 Comparison of subjectively estimated and objectively measured CTT. The average subjectively estimated CTTs are compared to the average objective kinematic measurements per specimen. CTT cranial tibial translation.

cruciate ligament rupture or rupture of both cruciate ligaments as "intact" or "unstable" in vitro. However, there was a considerable decrease in sensitivity (69%) and specificity (75%) when the CD was employed to differentiate between the pathological conditions. This indicates that even though participants were able to detect instability, they were unable to identify its origin.[31] In our study, the CD was able to accurately classify limbs as INTACT or CCLD, which is in line with the results of the study by Might et al. Due to the different study designs, no conclusion can be drawn upon the CDs ability to differentiate between CCLR and other ligamentous injuries of the stifle joint based on our results. In another study, Carobbi and Ness evaluated conscious dogs with naturally occurring CCLR and found a sensitivity as low as 60% for CD and 64% for TCT.[7] Under general anesthesia, sensitivity improved substantially (up to 92% for CD and 88% for TCT).[7] Specificity was 100% for both tests in both conditions.[7] This study also included dogs with partial CCLR and duration of lameness before testing was unknown. This might explain the reduced sensitivity compared to our results, as secondary periarticular fibrosis and osteoarthritic changes reduce stifle joint instability.[32] Considering this and the excellent interobserver agreement of tibial translation, it appears likely that other factors, such as presence of pain, periarticular fibrosis, or partial CCL tears, instead of interobserver variability, impair test accuracy in vivo.

The findings in our study surprisingly showed, opposed to the second part of our third hypothesis, that CTT was subjectively estimated with excellent reliability. A possible explanation for this is that in our experimental set up resembling acute CCLR there was either minimal (INTACT) or high degree of instability (CCLD), which enabled the observers to make an informed guess.

Despite this limitation, our results suggest that a grading scheme of laxity could be developed for dogs with acute complete CCLR.[12–14] Once validated, a grading system would improve the comparability of test results between observers and institutions. Also, in human medicine, specific treatment guidelines have been developed based on the grading of the pivot shift and Lachman test.[33,34] Therefore, a grading system for MLTs in veterinary medicine could become an essential tool in treatment decision-making, for example, when to add an extraarticular augmentation after TPLO.

The similarity of our results to those of other studies evaluating kinematics of canine CCLR validates our model as a novel method for testing kinematics in the CCL-deficient stifle.[28–30] Our testing setup was developed based on a previously reported setup for human knees.[35] It was built from 3D-printed components with a load cell seated between the specimen and the testing fixture. This design makes it very versatile and easily replicated. As no material testing machine is required, it provides a cost-effective and simple solution for performing future stifle kinematics studies. The templates to 3D-print the fixture are available on request from the corresponding author.

Limitations of this study include the small subset of specimens, the low number of observers per level of experience and the use of cadaveric hind limbs. Also, CCL-deficiency was mimicked by arthroscopic transection of the CCL instead of using limbs with naturally occurring CCLR. Clinical relevance of our results must therefore be confirmed in subsequent in vitro or in vivo studies with dogs suffering from chronic CCLR.

5 | CONCLUSION

Our results showed that the CD, the TCT and the newly introduced TPCT are accurate and reliable diagnostic tests in our model resembling acute CCLR. Despite variation in rotation and kinetics between observers, interobserver agreement of CTT was excellent. Following the development in human medicine, the establishment of a grading system could improve accuracy in vivo, as the magnitude of CTT was estimated with excellent agreement in our experimental setting. The TPCT seems to be promising and might have potential for the assessment of subtle or rotational instabilities of the canine stifle joint. Further in vivo investigations involving dogs with naturally occurring CCLR are warranted to confirm our findings and to validate the TPCT for a broader spectrum of CCL disease scenarios.

AUTHOR CONTRIBUTIONS

Lampart M, med vet: Involved in all steps of the study. Park BH, PhD, Husi, B, med vet and Pozzi A, MS, Prof Dr med vet: Contributed to study design, study execution, drafting and editing of the manuscript. Evans, R, PhD: Contributed to statistical analysis and drafting and editing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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