A pilot in vitro biomechanical comparison of locking compression plate fixation and kerf-cut cylinder fixation for ventral fusion of fourth and fifth equine cervical vertebrae

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Equine cervical vertebra, biomechanical testing, locking compression plate, kerf-cut cylinder

Summary
Introduction: The mechanical properties of equine cervical vertebrae joined by implants have not yet been reported. Locking compression plates (LCP) may provide a useful alternative system to the commonly used stainless steel kerf-cut cylinders (KCC) currently used for fixation of cervical vertebrae in horses.

Objectives: The objectives of this study were to establish a method for biomechanical testing of equine C4-C5 articulations and to compare the biomechanical properties of cadaveric spines stabilised with KCC and LCP.

Methods: Twenty-four equine cadaveric cervical spines were size measured from radiographs, and then randomly allocated to four groups. The C4-C5 articulation for each group was treated in the following way: group 1: KCC implanted, group 2: 8-hole 4.5/5.0 LCP implanted, group 3: 11-hole 3.5 LCP implanted and group 4: No implant. The specimens were fixed in resin and tested under four-point bending in ventral flexion until failure. Stiffness, maximum moment to failure and mode of failure were recorded for all specimens. General linear models were performed to analyse associations.

Results: All spines failed at the C4-C5 articulation. Failure however differed between groups with fractures in group 1, screw pull-out in groups 2 and 3, and disarticulation in group 4 being the common failure modes. The stiffness and maximum moment to failure of the KCC and LCP implanted spines were similar. Increasing age was significantly associated with decreasing moment to failure and increasing size was significantly associated with increasing moment to failure and stiffness.

Clinical significance: This study describes a successful technique for examining the biomechanical properties of equine cervical vertebrae. The LCP constructs had similar mechanical properties to the KCC which may justify their use in clinical cases.

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Introduction
The surgical placement of implants to encourage bony fusion and stability between cervical vertebrae as a treatment of cervical spinal cord compression in horses was first reported in 1979 (1). Since then the technique has been performed widely in North America and progressively more so in Europe. It has been estimated that up to 2,000 horses have had cervical fusion surgery (2). The technique has progressed from implantation of a homologous bone dowel to the implantation of a stainless steel cylinder with bone graft (Coward Bagby basket) and to the more recent open-ended threaded stainless steel kerf-cut cylinder (KCC) (2, 3).

Clinical assessment following the procedures report varying success; the rate of return to ridden work has been shown to range from 43 % to 79 % (2, 4–8). However the complication rate associated with the procedure is high, with a fatality rate of 9 % in one report (6). The major reported complications associated with the techniques are ventral migration of implant and vertebral fractures.

Vertebral fusion techniques are commonly performed in humans as treatment for degeneration, traumatic instability and other conditions (9). Between 1994 and 1999 more than 80,000 lumbar interbody fusion cages were implanted across the world (10). Recently, locking plates have been used for trans-cervical fixation techniques in humans, the mechanical properties of which have been tested in a human cadaveric model (9, 11). A locking compression plate (LCP) was used...
moved to the level of the intertransversarii cervicis muscles from the C3 to C6 (disarticulated between C2-C3 and C6-C7) spinal segment; joint capsules were left intact. The specimens were placed in sealed plastic bags and frozen to –20°C.

Implants

Group 1: KCC\(^b\) with the following features were used: height 26mm, internal diameter 23 mm, external unthreaded diameter 26 mm, external threaded diameter 30 mm. Each implant had four evenly spaced rows of twelve 4 mm diameter circular holes.

Group 2: Standard 4.5/5.0 broad 8-hole LCP\(^a\) plates (Synthes reference 226.581) with six 5.0 mm, self-tapping, locking screws. Screw length ranged from 32–55 mm (Synthes ref VS502.032 to .055)

Group 3: Standard 3.5 broad 11-hole LCP plates\(^c\) (Synthes reference VP4045.11) with eight 3.5 mm, self-tapping, locking screws. Screw length ranged from 30–45 mm (Synthes ref VS303.032 to .045).

Study design

Using a random number generator\(^d\) the spines were randomly allocated equally into four groups: groups 1–3 with the implants described above and group 4 without any implant.

The length of the vertebral body and height of the cranial vertebral body were measured for C4 and C5 from calibrated radiographs of the specimens, as described elsewhere (13). Specimen length was defined as the combined lengths of C4 and C5. Specimen height was defined as the mean of the two vertebral body heights. Overall specimen size was defined as specimen length multiplied by specimen height.

The spinal specimens were thawed at room temperature (20–22\(^\circ\)C) over a period of 12 hours prior to implant placement and resin fixation subsequent to which they were refrozen as before.

Kerf-cut cylinder placement (group 1)

Specimens were secured on a wooden board with a 23 mm deep pad of dense foam placed under the dorsal aspect of the C4-C5 articulation. The intertransversarii cervicis musculature over the ventral surface of C4 and C5 was sharply dissected. The ventral process of C4 was removed using an osteotome allowing access to the intervertebral disc. The KCC were placed using specialised equipment to drill and tap a cylindrical implant site between the bodies of C4 and C5, and then screw the cylinder into place as described elsewhere (14). Each implantation was timed with a stop watch from initial incision to end of implantation.

Locking compression plate placement (groups 2 and 3)

Specimens were secured and prepared as described for group 1 as far as removal of the

\(^{b}\) Wilson tool and Manufacturing Co., Washington, USA

\(^{d}\) Excel 2003; Microsoft, USA

\(^{c}\) Ziehm Vision, Nürnberg, Germany

Materials and methods

Specimens

Twenty-four equine cervical spines were collected within 24 hours of death from cadaveric adult horses that were aged by dentition. Ponies and draught breeds were excluded. The major external musculature was re-
ventral process of C4. A drill with a 15 mm bit was used to remove material from the ventral half of the intervertebral disc under radiographic guidance. The LCP were contoured to appose the ventral midline of C4 and C5. Screws were placed under radiographic guidance. For group 2: 5.0 mm locking, self-tapping screws were applied to the screw holes (numbered sequentially from cranial to caudal on the plate) in the following order: 3, 6, 1, 8, 2, 7 (the two centre holes were left empty). For group 3: 3.5 mm locking, self tapping screws were applied to the screw holes (numbered sequentially from cranial to caudal on the plate) in the following order: 4, 8, 1, 11, 2, 10, 3, 9 (the three centre holes were left empty). For both groups, screw placement was performed ensuring that screws did not interfere with any articulation or the spinal canal. All plates were applied in the same fashion using standard AO techniques relative to LCP placement, by the same clinician (CJL) for all 12 spines. Each implantation was timed with a stop watch from initial incision to end of implantation.

**Spine fixation**

Two (4 mm thread, 50 mm length) coated, high-grade steel screws were placed in a cranio-medio-caudal direction across the centre of each of the articular process joints between C3-C4 and C5-C6. Two (5 mm thread, 100 mm length) hardened, zinc plated, steel screws were placed in a cranio-ventral-caudodorsal direction in a sagittal plane across the intervertebral disc between C3-C4 and C5-C6.

**Resin fixation**

Specimens were then supported over a specially designed 15 cm³ cuboid mould and fixed in polyester resin to the level of the necks of the cranial articular processes of C4 cranially, and to the level of the necks of the caudal articular processes of C5 caudally in order to include the C3-C4 and the C5-C6 articulations in the resin. Resin fixation of both ends took less than six hours. The top inside edges of the resin blocks were trimmed to allow greater movement relative to the inner support points. A radiograph of a spine fixed with screws and resin is shown in Figure 1. Specimens were thawed at room temperature (20–22°C) for 12 hours prior to testing, and during testing they were kept moist with water. The time from collection to test period (freezer time) was limited to a maximum of three months.

**Biomechanical testing**

The resin blocks of the specimens were secured in steel boxes. Specimens were placed in a four-point bending fixture within a materials testing machine (Fig. 2). The outer support points were located on centrally placed pivot wheels on the outside of the steel boxes holding the resin. The outer support span was measured for each test specimen. The inner loading span was 100 mm in all cases. The inner loading points were applied equidistant on either side of the C4-C5 articulation. The inner support points consisted of 10 mm thick metal plates that were rounded on the C4 and wedge shaped on the C5 contact edges, and had 20 mm wide x 40 mm deep central sections removed, which gave clearance for the plate ventrally. The inner contact points differed in shape because it was thought that the wedge shaped point might prevent movement in a cranial caudal plane, whilst the rounded contact would allow hori-
Data analysis

Moment-angular displacement (4-point bending) (15) curves were constructed for each test. The slope of a simple linear regression of the linear (elastic) portion of each curve was used to determine stiffness in Nm/degree. Failure load and associated displacement were determined from the point of ultimate failure, that being the highest point reached on the load-deformation curve.

Bending moment $M$ was calculated using the following formula: $M = (FL)/2$.

- $F$ is the force (yield load or failure load) applied to the specimen,
- $L$ is $(S-T)/2$, and
- $S$ is the distance between the outer supports, and $T$ is the distance between the loading edges (Fig. 3).

Angular displacement $\phi$ was calculated by using the formulas: $\phi = \tan^{-1}(U/X)$ and $\phi_T = \phi_1 + \phi_2$.

- $\phi$ is the angular displacement,
- $U$ is the displacement of the LVDT,
- $X$ is the offset of the LVDT from the pivot support,
- $\phi_T$ is the total rotational displacement of the joint, and
- $\phi_1$ and $\phi_2$ are the rotations at each support (Fig. 4).

Failure mode

Each specimen was examined thoroughly pre- and post-testing and the site of failure, when evident, was identified and recorded. Digital photographs of the test set-up were taken at the start and end of each test. Latero-lateral (62 kV, 10 mAs) and dorso-ventral (68 kV, 10 mAs) radiographic projections centred on the C4-C5 articulation were taken prior to testing using computed radiography. Latero-lateral, flexed (supported between blocks allowing the C4-C5 articulation to flex in a ventral direction) and dorso-ventral radiographic projections centred on the C4-C5 articulation were taken post-testing. When it was not possible to accurately identify the failure mode on the radiographs, implants were removed and computed tomography (CT) images were collected of the C4 and C5 vertebrae.

Statistical analysis

Mean (± SD) stiffness and moment to failure were calculated for each of the test groups. Two general linear models were undertaken, one with stiffness as the outcome, and one with moment to failure as the outcome; age, size and group were included as predictors and group was fitted as a random effect. The final models were specified using a step-wise algorithm based on Akaike's information criterion. As the sample size for this study was convenience-based, a post-hoc power test was conducted to assess the power of the study.

Table 1 Results of generalised linear models for stiffness and moment to failure.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness</td>
<td>Intercept</td>
<td>4.964</td>
<td>7.681</td>
<td>0.5251</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.693</td>
<td>0.459</td>
<td>0.1463</td>
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<tr>
<td></td>
<td>Size</td>
<td>0.304</td>
<td>0.142</td>
<td>0.0438*</td>
</tr>
<tr>
<td>Moment to failure</td>
<td>Intercept</td>
<td>116.609</td>
<td>110.030</td>
<td>0.3013</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-14.281</td>
<td>6.583</td>
<td>0.0417*</td>
</tr>
<tr>
<td></td>
<td>Size</td>
<td>5.689</td>
<td>2.030</td>
<td>0.0107*</td>
</tr>
</tbody>
</table>

*Significant at P<0.05

Adjusted $R^2 = 0.2185$

Adjusted $R^2 = 0.1072$

Fig. 5 Images of the ventral aspect of the C4-C5 articulation after cutting with the core saw. A: specimen in which the ventral C4 part of the bony pinnacle had fractured and been removed (white arrow). B = specimen in which the bony pinnacle is intact (black arrow).
calculation using the standard deviations calculated from the residuals of each fitted model was undertaken. Statistical significance was set at $P < 0.05$. Statistical analyses were undertaken using R (16) and post-hoc power calculation was undertaken using statistical software.\footnote{\textit{knquery} Advisor, version 6: Statistical Solutions, Saugus, MA, USA}

**Results**

**Specimens**

The cadaveric specimens were collected from horses of ages ranging from five- to 20-years-old and with a median age of 12 years. The final general linear models fitted age and size as predictor but group was dropped from both models (Table 1). Age was found to be significantly associated with moment to failure ($P = 0.042$) but not stiffness. It was found that for any given sized horse, an increase in age by one year will result, on average, in a decrease in moment to failure by 14.28 Nm.

The calculated length of specimens ranged from 16.2 to 22.8 cm with a median length of 18.3 cm. The calculated height of the specimens ranged from 2.6 to 4.2 cm with a median height of 3.3 cm. Calculated size ranged from 41.5 cm$^2$ to 95.1 cm$^2$ with a median of 62.2 cm$^2$. Size was found to be significantly associated with stiffness ($P = 0.044$) and moment to failure ($P = 0.011$). For a horse of any given age, an increase in size by 1 cm$^2$ will result, on average, in an increase in stiffness by 0.30 Nm/deg and an increase in moment to failure by 5.69 Nm. The results of generalised linear models assessing association between age and size and stiffness and moment to failure are shown in Table 1.

**Implant placement time**

**Kerf-cut cylinder placement**

Time taken for KCC placement decreased. The first implant took one hour 10 minutes, whilst placement of the sixth implant took 28 minutes. In one specimen, placement of the KCC resulted in fracture of a small cranial ventral part of the central bone pinnacle (the ventral part of the C4 vertebral body left in the centre of the KCC from cutting with the cylindrical core saw) (Fig. 5).

**Locking compression plate placement**

The time taken to place the LCP implants decreased. The first implant in group 2 took 50 minutes, whilst placement of the sixth implant took 24 minutes. The first implant in group 3 took 45 minutes, whilst placement of the sixth implant took 30 minutes. Two specimens (one from each of groups 2 and 3) required replacement of one screw with a longer one, following radiographic assessment.

There was not any significant difference between the three groups in the time taken for implant placement.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{moment_vs_angle_graph.png}
\caption{Moment vs angle graph from group 1 demonstrating initial shallow incline followed by steeper incline. Dashed line represents the stiffness of the construct, dotted circle represents the failure point. Nm = Newton metre, deg = degree.}
\end{figure}
Mechanical test results

Following placement in the test unit and prior to load application, ventral flexion of the C4–C5 articulation was appreciated in the spines without implants but not in groups 1–3.

In all tests, an initial period of lower stiffness was apparent on the moment-angular rotation graphs (Fig. 6).

A review of the moment to failure and stiffness for each of the groups are shown in Table 2 and represented graphically in Figures 7 and 8.

There was not any significant difference in the failure load or stiffness between any of the groups tested. The post-hoc power calculation revealed that, with 80% power to detect an effect based on the variance between means and at a significance of 0.05, 149 spines would be required for each group for the moment to failure model and over 2,000 would be required for the stiffness model. Based on these findings, to detect a difference between fixation method for groups of ten specimens, there would need to be a difference greater than 30% in either moment to failure or stiffness.

Failure modes

Group 1: KCC failure mode
Five of the six KCC constructs failed via fracture of the C5 vertebral body between the caudal aspect of the cylinder and the vertebral canal (Fig. 9). The other specimen failed by ventral migration of the cylinder. Two of the specimens were imaged in the CT scanner to confirm failure site; one was the spine in which KCC migration had occurred, and the other demonstrated a fracture line in a similar position to those observed in the other fractured specimens. The specimen that failed via migration of the KCC did not demonstrate any change in stiffness or moment to yield compared to the other KCC implanted specimens.

Group 2: 4.5 Broad LCP failure mode
All constructs failed via screw pull-out, which was most notable from C5 in four of the six specimens. Minimal plate bending was appreciated. In one specimen the cranial screw was bent. None of the spines fractured.

Group 3: 3.5 Broad LCP failure mode.
All but one of the constructs failed via screw pull-out. Screw pull-out occurred primarily from C5. Plate deformation occurred in all but one of the specimens. In three of the specimens, screw bending was observed, ranging from two to six screws. In the specimen in which screw pull-out did not occur, the plate, two cranial and all four caudal screws were deformed. The spine that failed without screw pull-out demonstrated a markedly higher stiffness (double that of any of the other spines in this group), though the plate did ultimately bend. None of the spines fractured.

Group 4: No implant failure mode.
All six spines without implants failed via disarticulation at C4–C5.

Discussion

This study reports a successful method for applying consistent load across a single equine cervical vertebral articulation. Multiple pilot studies were performed to ensure movement occurred only at the C4–C5 articulation. The anatomy of the cervical vertebrae makes fixation and application of load across a single articulation difficult. Resin fixation of the specimens of just C3 and C6, insertion of metal rods in the spinal canal across the C3–C4 and C5–C6 articulations and leaving C3–C4 and C5–C6 unfixed all led to inconsistent failure outside the C4–C5 articulation. Furthermore, load application through a variety of different load points, including adjustable inner support points and loading arms led to inconsistent contact and specimen failure. Three-point bending was successful, but necessitated load application on either C4 or C5 and this was deemed inferior to four-point bending for examination of the mechanical properties of the constructs at this articulation. The final test model required placement of screws across the C3–C4 and C5–C6 articulations. Radiographic projections in two planes confirmed that these screws did not impinge upon the constructs in any of the specimens, as they were abaxial and cranial or caudal to them. None of the spines fractured at sites associated with the screws used for fixation of these articulations, suggesting they did not have an adverse effect on the vertebral structure.

The results of the study suggest that the mechanical properties of the LCP constructs are comparable to those of the KCC. Although differences were observed in the mechanical properties between implants, these were not statistically significant, which is likely to be partially the result of variability in the specimens, the small sample size and consequently, the relatively large standard deviation for each of the groups. However, it was determined that unreasonably large sample sizes would be required to find a significant association between fixation technique and moment to yield or stiffness (149 and over 2,000 spines per group respectively) and as such implies that if an association were to exist, it is unlikely to be mechanically important. A limitation of the study is that the testing involved high-load application in only one plane, which may be comparable to forces acting on a horse’s neck during a fall, such as might occur during recovery from general anaesthesia, but does not emulate the forces acting on the neck during normal movement. Others have recognised single-cycle loading as a useful way to obtain information about the stability of implants (17, 18). Repetitive loading of spines at physiologically represen-
Fig. 7  Relation of group with respect to moment to failure. Centre line is the median; interquartile ranges and total ranges are represented by the box and whiskers respectively. White diamonds represent the mean.

Fig. 8  Relation of group with respect to stiffness. Centre line is the median; interquartile ranges and total ranges are represented by the box and whiskers respectively. White diamonds represent the mean.
Fig. 9 Post testing latero-lateral radiograph of the C4-C5 articulation from group 1 post-testing. Left is cranial, top is ventral. Black arrows indicate the caudal end of the fracture line, white arrow shows the fracture fragment from the cranial body of C5.

which occurred as the inner contact points contacted the specimen and any residual movement in the C4-C5 articulation was taken up. The consistent nature of this finding is encouraging as it adds weight to the repeatability of the testing.

Placement of KCC occurred with minimal complications, which reflects the experience of the clinician undertaking the placement. The complication during KCC placement of the fracture of ventral cranial part of the bone pinnacle (Fig. 5) had previously occurred during surgery performed by one of the authors on clinical patients without apparent ill effect, and as a result the specimen was included in this study. Implantation of LCP was straightforward. Contouring of the plates to the ventral surface of the vertebrae was simple once the ventral process of C4 had been adequately removed. Two spines required screw replacement, which was performed without difficulty and may highlight a benefit of the LCP system over the KCC, where revision implant placement is not feasible.

The rapid decrease in implantation time over the six specimens for each of groups 1–3 was primarily the result of operator experience (both surgeon and technicians). The comparable implantation time between KCC and LCP was encouraging, however access to the surgical site was far easier in these dissected specimens than it would be in vivo. Although implantation of eight screws takes longer than six, the comparable implantation times between groups 2 and 3 observed in this study was a reflection of the speed of screw placement once radiographic measurement had been performed and the fact that group 3 implants were placed when the implant technique had received considerable practice in the application.

The failure modes differed between the groups. The relatively consistent fracture of the cranial articular end of the body of C5 in group 1 was interesting. To the authors’ knowledge, this complication has not been previously reported. This may be because the spines were being placed under far higher loads in this testing than they experience in vivo, or because the fractures are relatively hard to see, requiring stressed radiographic projections to observe them in some of these cases. It is also possible that the instability in the articulation caused by these small fractures is sufficiently supported by the extrinsic musculature to allow fracture healing without causing a clinical problem.

None of the LCP constructs resulted in vertebral fracture though the failure mode differed between the 4.5/5.0 and the 3.5 constructs. Screw and plate bending was observed frequently in group 3 compared with the bending of just a single screw in group 2. This reflects the size difference and hence different mechanical properties of the screws and plates. All but one of the LCP constructs failed via screw pull-out, which commonly occurred from vertebra C5. This failure mode was likely the result of the direction of flexion, with the construct on the compression side of the joint. The spine that failed by plate and screw deformation alone demonstrated a higher stiffness than all the other implanted spines, suggesting that construct stiffness may be closely related to pull-out strength of the screws for the LCP constructs. Construct failure by screw pull-out would be highly undesirable in a clinical case, but may not preclude revision surgery and implant replacement (12). By re-positioning the plate more cranially, caudally or abaxially, new holes could be drilled or different sized plates or screws could be used. Failures of KCC implanted spines could also be revised, but if fractures had occurred at the implant site, an alternative technique such as the use of a ventrally placed plate might be required to supplement it.

The spines without implants all failed via disarticulation, and no fractures were appreciated in this group. This highlights one possible adverse effect of the KCC implant that weakens the vertebra and alters the load distribution across the articulation.

The use of cadaveric specimens does not accurately emulate conditions in vivo, in which external soft tissue structures provide stability and compression around the cervical articulations and healing mechanisms stabilise the implant site with new bone proliferation. However, through the use of a standardised technique in which the same external support structures were removed for all cases, it is hoped that the tested samples were comparable. Many other studies have used cadaveric specimens for preliminary work (9, 11, 17, 18) as useful information can be obtained without the risk of animal morbidity or mortality.
Quite a large amount of variation in stiffness and moment to failure was appreciated within the groups in this study. There are numerous possible causes for this. The use of cadaveric specimens introduced uncontrollable variability as the anatomy and physiological structure of the bone and soft tissues inevitably varies. Use of fresh specimens may have been preferable to frozen ones and due to the constraints on collection, freezer time did vary between specimens. However specimens were handled in a consistent fashion; previous studies have used frozen cadavers for biomechanical testing (20). It has been shown that freezing has little effect on the mechanical properties of bone (21) and one study using pig cervical vertebrae concluded that freezing did not result in any change in stiffness or displacement at failure (22). Although specimens were kept moist externally, some drying of internal tissue was likely, and drying has been shown to effect mechanical properties (23). In this study, specimens from younger horses were found to have significantly higher moment to failure than those from older horses. Equine bone microarchitecture has been shown to vary with age (24), which likely explains this finding. Age of specimens in this study was elucidated from age is entirely accurate (25) so the ability to accurately predict moment to failure from age is questionable. The association of increasing spine size with increasing moment to failure and stiffness is unsurprising.

Conclusions
This pilot study describes a technique for testing the equine C4–C5 articulation and provides some preliminary data regarding its mechanical properties. In this study group the mechanical properties of articulations fixed with the LCP construct were comparable in ventral flexion, to spines fixed with the KCC construct, which may justify LCP use in clinical cases. Future work could involve investigation of the mechanical properties of spines in different planes of bending, the effects of cyclical loading and extrinsic musculature and the investigation of alternate implants and implantation techniques.

References