Use of 3D Printing Technology to Create a Canine Simulator for Cerebrospinal Fluid Sampling at the Lumbar Subarachnoid Space

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Use of 3D-printing technology to create a canine simulator for cerebrospinal fluid sampling at the lumbar subarachnoid space.

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Abstract

Cerebrospinal fluid (CSF) sampling at the lumbar subarachnoid space (LSS) is technically challenging to learn. Currently, training relies on cadaver availability or performance in a clinical scenario. This study aims to develop and validate a low cost, high-fidelity simulator to train in this technique. Using three-dimensional printing technology, a model of the lumbosacral vertebral column of a healthy adult dog was produced. The model was augmented with synthetic materials and a fluidic system to replicate all procedural steps and permit successful collection of CSF. The simulator was validated by experts (n=4), who rated it highly across multiple criteria. Final year veterinary students were recruited to take part in practical sessions using either the simulator (n=16) or a cadaver (n=16). Performance was recorded for each student and feedback was obtained using an anonymous online survey. Student performance was similar between groups (p=0.2), with 87.5% and 68.75% of students in the simulator and cadaver group, respectively, successfully placing the needle into the LSS. All successful students in the simulator group were able to obtain a CSF sample, compared to none in the cadaver group. No difference in the number of attempts was detected between groups (p>0.99), with the majority of students taking more than 3 attempts. User experience was similar between groups, with 93.8% of students in each group rating the session as a positive learning experience. In summary, we demonstrate the validity of a novel, low-cost and anatomically precise simulator which can be used for teaching CSF sampling at the LSS.

Key words

CSF, three-dimensional printing, model, education, veterinary neurology, lumbar puncture

Abbreviations

CSF cerebrospinal fluid
LSS lumbar subarachnoid space
3D three-dimensional
CMC cerebellomedullary cistern
BCS body condition score
EVA ethylene-vinyl acetate
ABS acrylonitrile butadiene styrene
ECVN European College of Veterinary Neurology
IQR interquartile range
Introduction

Cerebrospinal fluid (CSF) sampling at the lumbar subarachnoid space (LSS) is technically challenging to learn.\(^1\) This procedure is commonly performed as part of the diagnostic workup of a neurological patient.\(^2\) Multiple attempts are required to develop competence in this technique, which demands familiarity with the anatomical landmarks and tactile cues of the procedure. Currently, training in this technique relies on the availability of ethically sourced cadavers or performance in a clinical setting. In the latter scenario, training on client-owned animals can be complicated by clinical time pressures and the potential to cause iatrogenic harm to the patient, which may result in a negative learning experience. Furthermore, inexperience in this procedure has been shown to correlate with the risk of obtaining a blood contaminated, and potentially non-diagnostic, sample.\(^3\) While cadavers represent invaluable teaching resources, ethical, financial and logistical factors often preclude their use.\(^4\)

Additionally, yielding CSF in a cadaver can be challenging unless the procedure is performed immediately post-mortem, making successful performance of the procedure difficult to quantify. The development of alternative teaching models which resolve these issues would therefore benefit users wishing to train in this technique.

The use of training simulators in the veterinary curriculum is growing in popularity.\(^5,6\) Simulators allow repetitive practice and permit users to optimise their technique in a safe learning environment.\(^7\) Recent studies have demonstrated that training on simulators can improve students’ confidence, performance and learning ability, across a number of practical veterinary skills.\(^5-16\) Furthermore, recent meta-analyses have found that learning outcomes and proficiency are equivalent or greater in veterinary students taught using simulators versus those taught with traditional teaching models.\(^5,6\) However, a significant challenge in simulator design is the ability to accurately replicate the physical and functional characteristics of a living patient.\(^17,18\) To overcome this, others have integrated three-dimensional (3D) printing into simulator design.\(^11\) Three dimensional printing technology allows fast and precise reconstruction of anatomical specimens in a cost-effective manner.\(^16,19\) In human medicine, 3D-printed vertebral models have been incorporated into the design of novel simulators for anaesthesiology trainees to practice lumbar punctures and neuraxial blocks.\(^20-24\) In veterinary medicine, a similar model has recently been validated for teaching CSF sampling at the cerebellomedullary cervical junction (CMC).\(^11\) However, a simulator for teaching this procedure at the LSS has not yet been described.

The aim of this study was to use 3D-printing technology to design and validate a low-cost, high-fidelity simulator which accurately replicates all stages of the CSF sampling procedure at the LSS, and to compare the use of the simulator to a cadaver when teaching novice users this technique. We hypothesized that markers of performance would be similar between users trained on a cadaver or the simulator. We further predicted that user experience would be higher in those users trained on the simulator, which was designed to allow successful collection of CSF.
Materials and Methods

An outline of the study design is provided in Figure 1.

Ethical approval

This project was approved by the Human and Veterinary Ethics Research Committee at the Royal (Dick) School of Veterinary Studies (reference numbers: HERC 570-20, VERC 104-20).

Production of a 3D model of the lumbosacral vertebral column from CT data

The imaging database at the Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, was searched for computed tomography (CT) images of medium-sized dogs with normal lumbosacral vertebral columns. Computed tomography images (Siemens SOMATOM Definition AS; Siemens AG, Munich, Germany) of the lumbosacral vertebral column and iliac crests of a 28.7kg Bearded Collie (body condition score [BCS] 5/9) were chosen for 3D-printing. The CT images were initially processed using OsiriX DICOM Viewer software (Pixmeo SARL, Switzerland) using the 3D surface rendering tool and exported as stereolithography (.stl) files. These files were assembled and modified (to remove artefacts and ensure integrity of the anatomical landmarks) using Rhinoceros 3D software (Robert McNeel & Associates, Washington, USA). The angle of the lumbosacral vertebral column was adjusted into a slightly flexed position to simulate the position of patients undergoing CSF sampling at the LSS (Figure 2). The digital volume was subsequently exported into GrabCAD Print (Stratasys Ltd, Rehovot, Israel) slicing software in order to calculate the toolpaths and support structures required for printing. The final model, comprising L2 to the sacrum and iliac crests, was printed on a Dimension Elite 3D printer (Stratasys, Rehovot, Israel) using acrylonitrile butadiene styrene (ABS) (cartridge type P430, Stratasys Ltd) and a proprietary support material (cartridge type P400SR, Stratasys Ltd) (Figure 3). The total print time was 44 hours and 35 minutes.

Simulator fabrication

The simulator was created using materials similar to those described in human studies. All ingredients, manufacturers/suppliers, and costs are provided in the Supplementary Information. To facilitate the flow of cerebrospinal fluid through the model, 9mm diameter latex tubing was inserted through the vertebral canal of the 3D-printed model (Figure 3B). To replicate the ligamentum flavum, small slots were cut into a thin strip of 1.5mm thin EVA foam to allow placement over the spinous processes along the length of the model (Figure 3C). The soft tissues (epaxial musculature and subcutaneous fat) were recreated using a 15% ballistic gel according to the manufacturer’s instructions. Briefly, 300g gel powder was mixed with 1.7 litres of cold water. The mixture was refrigerated for 2 hours and subsequently heated to 39 degrees Celsius to form a liquid gel. The model, with tubing and ligamentum flavum in situ, was placed inside a custom-made plastic mould, which was created by cutting a commercially available manrose pipe in half (Figure 3D). The ends of the pipe were sealed with duct tape. The model was submerged with liquid ballistic gel and refrigerated for 24-hours. Once the gel had set (Figure 3E) the mould was removed. Prior to practical sessions, the final model (Figure 3F) was placed inside a commercial life-sized toy dog at the anatomically correct level. The toy dog was modified such that a small area of fabric was removed and replaced with synthetic skin at the site of sampling at the lumbar subarachnoid space (Figure 4A). The anterior portion of the latex tubing was connected to a 1 litre bag of 0.9% saline via a fluid administration set. Once the latex tubing was filled with saline, the posterior end of the latex tubing was clamped with a pair of artery forceps (Figure 4B). The fluidic system allowed flow of CSF following successful performance of the procedure. It is important to note that the ballistic gel is a perishable material. Therefore, to minimise degradation the model was removed from the toy dog and stored in a refrigerator between sessions. Furthermore, following multiple needle passes the gel will eventually lose its integrity. To overcome this, the ballistic gel can be peeled away from the 3D-printed model, re-melted and moulded back onto the model using the previously described steps. However, if an extended period of time (e.g., >72 hours) will pass between uses, we recommend that a fresh ballistic gel is made.

Model validation

Following fabrication, the model was validated by neurology clinicians (n=4, 1 European College of Veterinary Neurology [ECVN] diplomate and 3 ECVN residents) experienced in performing CSF sampling at the LSS. Clinicians were individually invited to perform the CSF sampling procedure on the simulator. Subsequently, they were asked to complete an anonymous online survey rating the simulator using a 5-point Likert scale (1 = ‘strongly disagree’, 2 = ‘disagree’, 3 = ‘neutral’, 4 = ‘agree’, 5 = ‘strongly agree’) against multiple criteria relating to its appearance, feel (compared to a living patient) and suitability for teaching (Table 1).
Final year veterinary students at Royal (Dick) School of Veterinary Studies were invited to take part in a practical session to practice CSF sampling at the LSS site. Students were excluded if they had any previous experience of performing CSF sampling. Prior to the practical session, students were asked to watch a 15-minute-long presentation detailing the theory behind CSF sampling, the anatomical landmarks and a video demonstrating the technique in a living patient. Students were then randomly allocated to the cadaver or simulator group to practice CSF sampling at the LSS. The practical sessions were performed on a one-to-one facilitator-to-student basis. The session facilitator (M.M.) recorded student performance across multiple criteria defined in Table 2. If the site for needle insertion (spinoous process of L6) was incorrectly identified, the student was corrected prior to continuing with the procedure. In the simulator group, correct needle placement was confirmed by witnessing the flow of CSF. In the cadaver group, students were asked to inform the facilitator when they thought the needle was in the LSS. The facilitator confirmed correct placement by manoeuvring the needle to gauge needle location and recorded whether accurate placement had been achieved (“yes”, “no” or “not sure”). If after 3 attempts, students were not successful, guidance was provided by the session facilitator. Successful performance was defined as placement the needle into the LSS, regardless of number of attempts or whether assistance was required. The following qualitative data was collected: number of attempts (less than 3 vs 3 or more); correct identification of the L6 spinous process (yes/no); successful placement of the needle into the LSS (yes/no/not sure) and successful collection of a CSF sample (yes/no); for individual students in each group. Following the practical session, students were asked to complete an anonymous online survey rating their experience with the cadaver or simulator across multiple criteria using a 5-point Likert scale (1 = ‘strongly disagree’, 2 = ‘disagree’, 3 = ‘neutral’, 4 = ‘agree’, 5 = ‘strongly agree’) (Table 3 and 4). Qualitative data and Likert scale ordinal data were collated and compared between groups to test our hypotheses.

Statistical analysis

Normality of quantitative variables was assessed using a Shapiro-Wilk Test and found to be non-parametric in distribution. Likert scale ordinal data were presented using descriptive statistics i.e. median and interquartile range. Likert scale ordinal data were compared using Mann-Whitney test. Qualitative data was compared using chi-squared or Fisher’s Exact Test. All statistical testing was performed using GraphPad Prism 8.4.2 for macOS (GraphPad Software, San Diego, California USA, www.graphpad.com). Results were considered statistically significant when p<0.05.
Results

Model construction and cost

The total production cost of the simulator was £173.87 ([Supplementary information](#)). This total excludes costs associated with the purchase or maintenance of a 3D printer and software. The total construction time was 70 hours and 5 minutes. This included 68 hours and 35 minutes hands off time (44 hours and 35 minutes for 3D printing and 24 hours for the gel to set) and approximately 1 hour and 30 minutes hands on time (installing the tubing, addition of ligamentum flavum, preparation of the mould, melting the gel, modifying the soft dog toy, installing the model into the dog toy and setting up the fluidic system).

Expert validation

Feedback from the experts (*n* = 4) was positive, with the model scoring highly (median >4) across all criteria. All experts “agreed” (*n* = 2) or “strongly agreed” (*n* = 2) that, compared to a cadaver, the simulator was suitable for teaching CSF sampling at the LSS ([Table 1](#)).

Student performance

Students in the simulator group were more likely to identify the correct site for needle insertion than those in the cadaver group (*n* = 16/16 simulator group, *n* = 5/16 cadaver group, *p* = <0.0001, [Table 2](#)). Once the correct site for needle insertion was confirmed by the facilitator, student performance was similar between groups, with 87.5% and 68.75% of students in the simulator and cadaver group, respectively, successfully placing the needle into the LSS (*n* = 14/16 in the simulator group, *n* = 11/16 in the cadaver group, *p* = 0.2, [Table 2](#)). In the cadaver group, it was not possible for the facilitator to determine whether one student had correctly placed their needle into the LSS or not. All successful students in the simulator group were able to obtain a CSF sample, compared with none in the cadaver group (*p* < 0.0001, [Table 2](#)). No difference in the number of attempts was detected between groups (*p* > 0.99), with the majority of students taking more than 3 attempts (i.e., requiring assistance) to place the needle into the LSS ([Table 2](#)).

Student self-assessment and experience

Between groups, there were no statistically significant differences in the students’ self-reported ability to perform each step of the CSF sampling procedure at the LSS ([Table 3](#)). Student experience was also similar between groups, with median values across all criteria falling into the “strongly agree” or “agree” category ([Table 4](#)). Importantly, 93.8% (*n* = 15/16) of students in each group rated the practical session as a positive learning experience ("strongly agree” or “agree”). The majority of students “agreed” (cadaver group: 4/16; simulator group: 7/16) or “strongly agreed” (cadaver group: 10/16; simulator group: 4/16) that they “would feel confident to attempt this procedure on a living patient under direct supervision”. Interestingly, the proportion of students that strongly agreed with this statement was higher in the cadaver group (*n* = 10/16) compared to the simulator group (*n* = 4/16).
Discussion

Simulator training is becoming increasingly recognised as a valuable teaching method within veterinary medical education. In this study, we drew from simulator design in human studies and used 3D-printing technology to create the first reported canine simulator for CSF sampling at the LSS. We describe the production of the simulator and show that this can be performed at low cost. Our data suggests that the simulator accurately replicates each step of the CSF sampling procedure and represents an effective teaching aid when compared to traditional teaching methods, i.e., cadaver training. We propose that the simulator will make a useful teaching resource for undergraduate and postgraduate (i.e., internship and residency) veterinary training programs and provide detailed methodology to allow it to be reproduced by other institutions.

Human studies have demonstrated that simulator training can promote skill transfer to a clinical setting and reduce complication rates during performance of clinical or surgical procedures. However, the functional and physical fidelity of simulators often falls short of the real life scenario, which could compromise the acquisition of psychomotor skills required to perform a specific procedure. With the advent of 3D-printing technology, it is now possible to produce the anatomically precise components required to simulate clinical procedures that rely on defined anatomical landmarks. In contrast to human medicine, there are very few reports in veterinary medicine which have used 3D-printing technology to produce anatomical models or training simulators. We propose that ongoing integration of 3D-printing technology into simulator design will improve their fidelity resulting in a reduced requirement for cadavers (and the financial, logistical and ethical implications of their use) within the veterinary curriculum.

Overall, this study did not find a difference in user performance between students trained on the simulator or the cadaver, supporting our initial hypothesis. We found that students in the cadaver group were more likely to incorrectly identify the appropriate site for needle insertion, suggesting that the anatomical landmarks were easier to identify in the simulator model. A similar finding was reported in a study by Langebæk et al (2020) who used comparable techniques to produce a simulator for CSF sampling at the CMC. In our study, the disparity in the ability to palpate the anatomical landmarks between simulator and cadaver may be explained by the subtle difference in BCS, individual variation in lumbosacral anatomy or suboptimal replication of the soft tissue structures. However, the ability to palpate the anatomical structures with ease in our model represents an advantage for inexperienced users, who would benefit from familiarising themselves with the anatomical landmarks in a standardised manner prior to performing the procedure in the more varied population presented in clinical practice. Interestingly, despite the ability to palpate anatomical landmarks clearly and collect a CSF sample, students in the simulator-trained group did not feel as confident as students in the cadaver-trained group to attempt the procedure in a living patient under supervision, although this result was not significant. In the study by Langebæk et al (2020), students preferred training on a cadaver over the simulator. In contrast to our own study design, students in the study by Langebæk et al (2020) were given the opportunity to perform the procedure using both a cadaver and the simulator. These students reported that they found the CSF sampling procedure to be less difficult on the simulator and raised concern that they may become overly confident in the procedure if trained using this method alone. Furthermore, the students perceived that the anatomical variation of cadavers provided them with a better representation of the clinical scenario. Taking these findings together, it seems likely that optimal training in this technique would benefit from both methods of teaching - using the simulator to familiarise oneself with the procedural steps of the technique prior to performance in a cadaver and subsequently a living patient.

Human medical and veterinary educational reviews on simulator-based training discuss how simulators designed to give feedback can enhance the learning experience and facilitate self-directed learning. As such, our simulator was specifically designed to provide feedback to students during performance of the procedure, by allowing physical collection of a CSF sample. We hypothesized that this feature would enhance user experience in the simulator group compared to the cadaver group. However, our data did not support this hypothesis – students in both groups rated their experiences equally. This finding may have been confounded by the fact that, despite not being able to obtain a CSF sample in the cadaver group, the session facilitator provided verbal feedback to students on whether they had been successful in placing the needle into the LSS. In most cases, it was possible for the session facilitator to determine if the needle was placed into the LSS. However, this can be time-consuming and in a self-directed session it would be challenging for a novice user to determine if they had successfully performed the procedure. In contrast, the simulator provides immediate feedback and indication of success to the user. For this reason, we predict that a difference in user experience would be detected if the simulator was tested against a cadaver in a self-directed scenario.

In summary, 3D-printing technology has the potential to enhance the anatomical accuracy of veterinary simulators, reducing the reliance on cadavers in veterinary medical education. Simulators provide the opportunity for users to undertake the standardised and repetitive training required to reinforce their clinical skills in a safe environment. Further research is required to understand the role of such simulators in self-directed learning.
This study has some limitations including the recruitment of students on a volunteer basis, which may have introduced volunteer bias into the study.31 Furthermore, while the students had not performed the CSF sampling procedure before, it is possible that some students had witnessed the procedure during their clinical rotations. Finally, the experts invited to validate the simulator were members of our own institution, which may have resulted in biased feedback on the simulator design. Simulator design was sufficient for the aims of this study. However, certain features of the simulator would benefit from further optimisation. For example, with the existing fluidic system it was difficult to maintain a consistent speed of CSF flow between users due to pressure changes inside the tubing as fluid was removed with each subsequent use. The authors do not feel that this feature influences the ability to effectively learn the procedural steps involved in this technique. Furthermore, the current 3D-printed model lacks the flexibility of a vertebral column in vivo. Integrating flexible filaments into the vertebral articulations of the 3D-printed model would enhance the resilience and fidelity of the model.16 Future iterations of the model would benefit from optimising these features.

**Conclusions:**
This study describes the development and validation of a novel and anatomically precise simulator for training novice users to perform CSF sampling at the LSS, that can be easily reproduced at a low cost. We demonstrate that the simulator is comparable to the use of a cadaver for teaching this procedure to novice users during facilitated sessions. Further work is required to optimise simulator design and to investigate the role of the simulator in a self-directed learning scenario and to document the efficacy of skill transfer to a clinical setting. In the future, we envisage that the simulator could be repurposed for training in other advanced procedures, e.g., epidural anaesthesia techniques, and would encourage colleagues to optimise the simulator for such use.

**Acknowledgements**
The authors would like to thank all of the students and members of staff that volunteered to participate in this study. We would also like to thank the Principal Teaching Award Scheme (PTAS), who funded this project.

**References**


Tables

Table 1: Results of the expert validation survey (n=4)

<table>
<thead>
<tr>
<th>Question</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>The simulator was easy to use</td>
<td>4.5</td>
<td>4-5</td>
</tr>
<tr>
<td>The visual appearance of the simulator was realistic</td>
<td>4.5</td>
<td>4-5</td>
</tr>
<tr>
<td>The anatomical landmarks were accurate</td>
<td>4</td>
<td>4-4.75</td>
</tr>
<tr>
<td>Palpation of soft tissue and bony landmarks was realistic</td>
<td>4</td>
<td>4-4.75</td>
</tr>
<tr>
<td>Properties of needle insertion were similar to in a living patient</td>
<td>4</td>
<td>4-4.75</td>
</tr>
<tr>
<td>Appearance and flow of CSF was similar to in a living patient</td>
<td>5</td>
<td>4.25-5</td>
</tr>
<tr>
<td>The simulator is adequate, when compared to a cadaver, for the purpose of teaching the method of CSF sampling</td>
<td>4.5</td>
<td>4-5</td>
</tr>
</tbody>
</table>

1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree
CSF = cerebrospinal fluid, IQR = interquartile range

Table 2: Student performance as recorded by the session facilitator

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Number of students</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct identification of the L6 spinous process</td>
<td>Cadaver group</td>
<td>Simulator group</td>
</tr>
<tr>
<td>Successful insertion of the needle into the LSS</td>
<td>11/16</td>
<td>14/16</td>
</tr>
<tr>
<td>Successful collection of a CSF sample</td>
<td>0/16</td>
<td>14/16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of attempts</th>
<th>Cadaver group</th>
<th>Simulator group</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 3</td>
<td>4/11</td>
<td>6/14</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>3 or more</td>
<td>7/11</td>
<td>8/14</td>
<td></td>
</tr>
</tbody>
</table>
**Table 3: Student performance as recorded via the self-assessment survey (n = 16 per group)**

<table>
<thead>
<tr>
<th>Question</th>
<th>Cadaver</th>
<th>IQR</th>
<th>Simulator</th>
<th>IQR</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I was able to position the cadaver/model easily for CSF collection</td>
<td>5</td>
<td>5-5</td>
<td>5</td>
<td>5-5</td>
<td>p = 0.30</td>
</tr>
<tr>
<td>I was able to palpate the anatomical landmarks</td>
<td>4</td>
<td>4-5</td>
<td>4</td>
<td>4-5</td>
<td>p = 0.85</td>
</tr>
<tr>
<td>I was able to identify the location for needle insertion</td>
<td>4</td>
<td>4-5</td>
<td>4</td>
<td>4-5</td>
<td>p = 0.98</td>
</tr>
<tr>
<td>I was able to insert the needle through the skin and muscle easily</td>
<td>5</td>
<td>5-5</td>
<td>4</td>
<td>4-5</td>
<td>p = 0.23</td>
</tr>
<tr>
<td>I was able to determine when the needle was in the correct location to collect CSF</td>
<td>4</td>
<td>3.25-4.75</td>
<td>4</td>
<td>3.25-5</td>
<td>p = 0.82</td>
</tr>
</tbody>
</table>

* Mann-Whitney test  
1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree  
CSF = cerebrospinal fluid, IQR = interquartile range

**Table 4: Student experience survey (n = 16 per group)**

<table>
<thead>
<tr>
<th>Question</th>
<th>Cadaver</th>
<th>IQR</th>
<th>Simulator</th>
<th>IQR</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I found this to be a positive learning experience</td>
<td>5</td>
<td>5-5</td>
<td>5</td>
<td>5-5</td>
<td>p = 0.70</td>
</tr>
<tr>
<td>I enjoyed this method of practising CSF sampling</td>
<td>5</td>
<td>5-5</td>
<td>5</td>
<td>5-5</td>
<td>p = 0.97</td>
</tr>
<tr>
<td>This session improved my understanding of CSF sampling technique</td>
<td>5</td>
<td>5-5</td>
<td>5</td>
<td>5-5</td>
<td>p = 0.65</td>
</tr>
<tr>
<td>I felt comfortable practising the technique using the cadaver/model</td>
<td>5</td>
<td>5-5</td>
<td>5</td>
<td>5-5</td>
<td>p = 0.31</td>
</tr>
<tr>
<td>I would feel confident to attempt this procedure on a living patient under direct supervision</td>
<td>5</td>
<td>4-5</td>
<td>4</td>
<td>3-4.75</td>
<td>p = 0.06</td>
</tr>
</tbody>
</table>

* Mann-Whitney test  
1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree  
CSF = cerebrospinal fluid, IQR = interquartile range

**Figure captions**

**Figure 1: Flow chart of the study design**
CSF = cerebrospinal fluid, LSS = lumbar subarachnoid space

**Figure 2: Preparation of CT images for 3D-printing**
A + B, Volume rendered 3D reconstruction of the lumbosacral vertebral column of a healthy dog.  
C, Digital model, amended in order to correct artefacts to ensure integrity of anatomical landmarks, and ensure patency of the vertebral canal and L5/L6 foramina during the printing process.  
D, Slight flexion applied to L3-L5 portion of the digital model to simulate the flexed position of the pelvic limbs during CSF sampling.

**Figure 3: Constructing the simulator using the 3D-printed model**
A, 3D-printed model of the lumbosacral vertebral column.  
B, Insertion of latex tubing into the vertebral canal to facilitate flow of ‘cerebrospinal fluid’.

Chi-squared test  
Fisher’s exact test. Significant p-values are highlighted in bold.  
CSF = cerebrospinal fluid; LSS = lumbar subarachnoid space.
Figure 4: Completed construction of the simulator

A. The final model (Figure 3F) is inserted inside a life-sized soft toy dog. Synthetic skin is placed at the appropriate level for CSF sampling at the LSS. A 1-litre bag of saline is attached to one end of the latex tubing via an administration set. The latex tubing is primed with saline. The other end of the latex tubing is clamped with artery forceps. An infusion pressure bag ensures a constant pressure within the latex tubing to allow flow of CSF.

B. Successful collection of CSF at the LSS using the simulator.