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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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61 Abstract 62

63 Cerebrospinal fluid (CSF) sampling at the lumbar subarachnoid space (LSS) is technically challenging to learn. 64 Currently, training relies on cadaver availability or performance in a clinical scenario. This study aims to develop 65 and validate a low cost, high-fidelity simulator to train in this technique. Using three-dimensional printing 66 technology, a model of the lumbosacral vertebral column of a healthy adult dog was produced. The model was 67 augmented with synthetic materials and a fluidic system to replicate all procedural steps and permit successful 68 collection of CSF. The simulator was validated by experts (n=4), who rated it highly across multiple criteria. Final 69 year veterinary students were recruited to take part in practical sessions using either the simulator (n=16) or a 70 cadaver (n=16). Performance was recorded for each student and feedback was obtained using an anonymous 71 online survey. Student performance was similar between groups (p=0.2), with 87.5% and 68.75% of students in 72 the simulator and cadaver group, respectively, successfully placing the needle into the LSS. All successful 73 students in the simulator group were able to obtain a CSF sample, compared to none in the cadaver group. No 74 difference in the number of attempts was detected between groups (p>0.99), with the majority of students taking 75 76 77 78 79 80 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
- 81 **Abbreviations**
- 82 83 CSF cerebrospinal fluid
- 84 LSS lumbar subarachnoid space
- 3D three-dimensional
- 85 86 CMC cerebellomedullary cistern
- 87 BCS body condition score
- 88 EVA ethylene-vinyl acetate
- 89 ABS acrylonitrile butadiene styrene
- 90 ECVN European College of Veterinary Neurology
- 91 IQR interquartile range

Introduction

92 93 94 Cerebrospinal fluid (CSF) sampling at the lumbar subarachnoid space (LSS) is technically challenging to learn.¹ 95 This procedure is commonly performed as part of the diagnostic workup of a neurological patient.² Multiple 96 attempts are required to develop competence in this technique, which demands familiarity with the anatomical 97 landmarks and tactile cues of the procedure. Currently, training in this technique relies on the availability of 98 ethically sourced cadavers or performance in a clinical setting. In the latter scenario, training on client-owned 99 animals can be complicated by clinical time pressures and the potential to cause iatrogenic harm to the patient, 100 which may result in a negative learning experience. Furthermore, inexperience in this procedure has been shown 101 to correlate with the risk of obtaining a blood contaminated, and potentially non-diagnostic, sample.³ While 102 cadavers represent invaluable teaching resources, ethical, financial and logistical factors often preclude their use.⁴ 103 Additionally, yielding CSF in a cadaver can be challenging unless the procedure is performed immediately post-104 mortem, making successful performance of the procedure difficult to quantify. The development of alternative 105 teaching models which resolve these issues would therefore benefit users wishing to train in this technique.

106 The use of training simulators in the veterinary curriculum is growing in popularity.^{5,6} Simulators allow 107 repetitive practice and permit users to optimise their technique in a safe learning environment.⁷ Recent studies 108 have demonstrated that training on simulators can improve students' confidence, performance and learning ability, 109 across a number of practical veterinary skills.⁸⁻¹⁶ Furthermore, recent meta-analyses have found that learning 110 outcomes and proficiency are equivalent or greater in veterinary students taught using simulators versus those 111 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 112 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, 113 114 115 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 116 117 recently been validated for teaching CSF sampling at the cerebellomedullary cervical junction (CMC).¹¹ However, 118 a simulator for teaching this procedure at the LSS has not yet been described.

119 The aim of this study was to use 3D-printing technology to design and validate a low-cost, high-fidelity 120 simulator which accurately replicates all stages of the CSF sampling procedure at the LSS, and to compare the 121 use of the simulator to a cadaver when teaching novice users this technique. We hypothesized that markers of 122 performance would be similar between users trained on a cadaver or the simulator. We further predicted that user 123 experience would be higher in those users trained on the simulator, which was designed to allow successful 124 collection of CSF.

126 Materials and Methods127

- 128 An outline of the study design is provided in Figure 1.129
- 130 *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).

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

135 The imaging database at the Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, was searched 136 for computed tomography (CT) images of medium-sized dogs with normal lumbosacral vertebral columns. 137 Computed tomography images (Siemens SOMATOM Definition AS; Siemens AG, Munich, Germany) of the 138 lumbosacral vertebral column and iliac crests of a 28.7kg Bearded Collie (body condition score [BCS] 5/9) were 139 chosen for 3D-printing. The CT images were initially processed using OsiriX DICOM Viewer software (Pixmeo 140 SARL, Switzerland) using the 3D surface rendering tool and exported as stereolithography (.stl) files. These files 141 were assembled and modified (to remove artefacts and ensure integrity of the anatomical landmarks) using 142 Rhinoceros 3D software (Robert McNeel & Associates, Washington, USA). The angle of the lumbosacral 143 vertebral column was adjusted into a slightly flexed position to simulate the position of patients undergoing CSF 144 sampling at the LSS (Figure 2). The digital volume was subsequently exported into GrabCAD Print (Stratasys 145 Ltd, Rehovot, Israel) slicing software in order to calculate the toolpaths and support structures required for 146 printing. The final model, comprising L2 to the sacrum and iliac crests, was printed on a Dimension Elite 3D 147 printer (Stratasys, Rehovot, Israel) using acrylonitrile butadiene styrene (ABS) (cartridge type P430, Stratasys 148 Ltd) and a proprietary support material (cartridge type P400SR, Stratasys Ltd) (Figure 3). The total print time 149 was 44 hours and 35 minutes.

150 151 Simulator fabrication

152 The simulator was created using materials similar to those described in human studies²⁰⁻²⁴. All ingredients, 153 manufacturers/suppliers and costs are provided in the Supplementary Information. To facilitate the flow of 154 'cerebrospinal fluid' through the model, 9mm diameter latex tubing was inserted through the vertebral canal of 155 the 3D-printed model (Figure 3B). To replicate the ligamentum flavum, small slots were cut into a thin strip of 156 1.5mm thin EVA foam to allow placement over the spinous processes along the length of the model (Figure 3C). 157 The soft tissues (epaxial musculature and subcutaneous fat) were recreated using a 15% ballistic gel according to 158 the manufacturer's instructions. Briefly, 300g gel powder was mixed with 1.7 litres of cold water. The mixture 159 was refrigerated for 2 hours and subsequently heated to 39 degrees Celsius to form a liquid gel. The model, with 160 tubing and ligamentum flavum in situ, was placed inside a custom-made plastic mould, which was created by 161 cutting a commercially available manrose pipe in half (Figure 3D). The ends of the pipe were sealed with duct 162 tape. The model was submerged with liquid ballistic gel and refrigerated for 24-hours. Once the gel had set 163 (Figure 3E) the mould was removed. Prior to practical sessions, the final model (Figure 3F) was placed inside a 164 commercial life-sized toy dog at the anatomically correct level. The toy dog was modified such that a small area 165 of fabric was removed and replaced with synthetic skin at the site of sampling at the lumbar subarachnoid space 166 (Figure 4A). The anterior portion of the latex tubing was connected to a 1 litre bag of 0.9% saline via a fluid 167 administration set. Once the latex tubing was filled with saline, the posterior end of the latex tubing was clamped 168 with a pair of artery forceps (Figure 4B). The fluidic system allowed flow of CSF following successful 169 performance of the procedure. It is important to note that the ballistic gel is a perishable material. Therefore, to 170 minimise degradation the model was removed from the toy dog and stored in a refrigerator between sessions. 171 Furthermore, following multiple needle passes the gel will eventually lose its integrity. To overcome this, the 172 ballistic gel can be peeled away from the 3D-printed model, re-melted and moulded back onto the model using 173 the previously described steps. However, if an extended period of time (e.g., >72 hours) will pass between uses, 174 we recommend that a fresh ballistic gel is made. 175

176 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**).

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186 *Cadaver requisition*

A size-matched (31.7kg Labrador, BCS 6/9) fresh cadaver was ethically obtained through the body memorial donation scheme at the Royal (Dick) School of Veterinary Studies. The cadaver was positioned for CSF sampling post-mortem (prior to the onset of rigor mortis) with the pelvic limbs in a flexed position. The same cadaver was used for all students assigned to the cadaver group over a week-long period. In between sessions, the cadaver was kept in a temperature-controlled cold store.

193 Study design

192

194 Final year veterinary students at Royal (Dick) School of Veterinary Studies were invited to take part in a practical 195 session to practice CSF sampling at the LSS site. Students were excluded if they had any previous experience of 196 performing CSF sampling. Prior to the practical session, students were asked to watch a 15-minute-long 197 presentation detailing the theory behind CSF sampling, the anatomical landmarks and a video demonstrating the 198 technique in a living patient. Students were then randomly allocated to the cadaver or simulator group to practice 199 CSF sampling at the LSS. The practical sessions were performed on a one-to-one facilitator-to-student basis. The 200 session facilitator (M.M.) recorded student performance across multiple criteria defined in Table 2. If the site for 201 needle insertion (spinous process of L6) was incorrectly identified, the student was corrected prior to continuing 202 with the procedure. In the simulator group, correct needle placement was confirmed by witnessing the flow of 203 CSF. In the cadaver group, students were asked to inform the facilitator when they thought the needle was in the 204 LSS. The facilitator confirmed correct placement by manoeuvring the needle to gauge needle location and 205 recorded whether accurate placement had been achieved ("yes", "no" or "not sure"). If after 3 attempts, students 206 were not successful, guidance was provided by the session facilitator. Successful performance was defined as 207 placement the needle into the LSS, regardless of number of attempts or whether assistance was required. The 208 following qualitative data was collected: number of attempts (less than 3 vs 3 or more); correct identification of 209 the L6 spinous process (yes/no); successful placement of the needle into the LSS (yes/no/not sure) and successful 210 collection of a CSF sample (yes/no); for individual students in each group. Following the practical session, 211 students were asked to complete an anonymous online survey rating their experience with the cadaver or simulator 212 across multiple criteria using a 5-point Likert scale (1 = 'strongly disagree', 2 = 'disagree', 3 = 'neutral', 4 = 213 214 '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. 215

216 Statistical analysis

217 Normality of quantitative variables was assessed using a Shapiro-Wilk Test and found to be non-parametric in 218 distribution. Likert scale ordinal data were presented using descriptive statistics i.e. median and interquartile

219 range. Likert scale ordinal data were compared using Mann-Whitney test.²⁵ Qualitative data was compared using

220 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.

224 225 Results

226 227 Model construction and cost

The total production cost of the simulator was $\pounds 173.87$ (Supplementary information). This total excludes costs 228 associated with the purchase or maintenance of a 3D printer and software. The total construction time was 70 229 hours and 5 minutes. This included 68 hours and 35 minutes hands off time (44 hours and 35 minutes for 3D-230 printing and 24 hours for the gel to set) and approximately 1 hour and 30 minutes hands on time (installing the 231 tubing, addition of ligamentum flavum, preparation of the mould, melting the gel, modifying the soft dog toy, 232 installing the model into the dog toy and setting up the fluidic system). 233

234 Expert validation

235 Feedback from the experts (n = 4) was positive, with the model scoring highly (median >4) across all criteria. All 236 experts "agreed" (n=2) or "strongly agreed" (n=2) that, compared to a cadaver, the simulator was suitable for 237 teaching CSF sampling at the LSS (Table 1). 238

239 Student performance

240 Students in the simulator group were more likely to identify the correct site for needle insertion than those in the 241 cadaver group (n = 16/16 simulator group, n = 5/16 cadaver group, p = <0.0001, Table 2). Once the correct site 242 243 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 244 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 245 was not possible for the facilitator to determine whether one student had correctly placed their needle into the LSS 246 or not. All successful students in the simulator group were able to obtain a CSF sample, compared with none in 247 the cadaver group (p < 0.0001, **Table 2**). No difference in the number of attempts was detected between groups 248 (p > 0.99), with the majority of students taking more than 3 attempts (i.e., requiring assistance) to place the needle 249 into the LSS (Table 2).

250

Student self-assessment and experience

251 252 Between groups, there were no statistically significant differences in the students' self-reported ability to perform 253 each step of the CSF sampling procedure at the LSS (Table 3). Student experience was also similar between 254 255 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 256 experience ("strongly agree") or "agree"). The majority of students "agreed" (cadaver group: 4/16; simulator 257 group: 7/16) or "strongly agreed" (cadaver group: 10/16; simulator group: 4/16) that they "would feel confident 258 to attempt this procedure on a living patient under direct supervision". Interestingly, the proportion of students 259 that strongly agreed with this statement was higher in the cadaver group (n = 10/16) compared to the simulator 260 group (n = 4/16).

263 Discussion 264

265 Simulator training is becoming increasingly recognised as a valuable teaching method within veterinary medical 266 education.⁵ In this study, we drew from simulator design in human studies and used 3D-printing technology to 267 create the first reported canine simulator for CSF sampling at the LSS. We describe the production of the simulator 268 and show that this can be performed at low cost. Our data suggests that the simulator accurately replicates each 269 step of the CSF sampling procedure and represents an effective teaching aid when compared to traditional teaching 270 methods, i.e., cadaver training. We propose that the simulator will make a useful teaching resource for 271 undergraduate and postgraduate (i.e., internship and residency) veterinary training programs and provide detailed 272 methodology to allow it to be reproduced by other institutions.

273 Human studies have demonstrated that simulator training can promote skill transfer to a clinical setting²⁶ 274 and reduce complication rates during performance of clinical or surgical procedures.^{27,28} However, the functional and physical fidelity of simulators often falls short of the real life scenario,¹⁷ which could compromise the 275 276 acquisition of psychomotor skills required to perform a specific procedure. With the advent of 3D-printing 277 technology, it is now possible to produce the anatomically precise components required to simulate clinical procedures that rely on defined anatomical landmarks.^{16,20-24} In contrast to human medicine, there are very few 278 279 reports in veterinary medicine which have used 3D-printing technology to produce anatomical models^{16,19,29} or 280 training simulators.¹¹ We propose that ongoing integration of 3D-printing technology into simulator design will 281 improve their fidelity resulting in a reduced requirement for cadavers (and the financial, logistical and ethical 282 implications of their use)⁴ within the veterinary curriculum.

283 Overall, this study did not find a difference in user performance between students trained on the simulator 284 or the cadaver, supporting our initial hypothesis. We found that students in the cadaver group were more likely to 285 incorrectly identify the appropriate site for needle insertion, suggesting that the anatomical landmarks were easier 286 to identify in the simulator model. A similar finding was reported in a study by Langebæk et al (2020) who used 287 comparable techniques to produce a simulator for CSF sampling at the CMC. In our study, the disparity in the 288 ability to palpate the anatomical landmarks between simulator and cadaver may be explained by the subtle 289 difference in BCS, individual variation in lumbosacral anatomy or suboptimal replication of the soft tissue 290 291 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 292 landmarks in a standardised manner prior to performing the procedure in the more varied population presented in 293 clinical practice.⁷ Interestingly, despite the ability to palpate anatomical landmarks clearly and collect a CSF 294 sample, students in the simulator-trained group did not feel as confident as students in the cadaver-trained group 295 to attempt the procedure in a living patient under supervision, although this result was not significant. In the study 296 by Langebæk et al (2020), students preferred training on a cadaver over the simulator. In contrast to our own study 297 design, students in the study by Langebæk et al (2020) were given the opportunity to perform the procedure using 298 both a cadaver and the simulator. These students reported that they found the CSF sampling procedure to be less 299 difficult on the simulator and raised concern that they may become overly confident in the procedure if trained 300 using this method alone. Furthermore, the students perceived that the anatomical variation of cadavers provided 301 them with a better representation of the clinical scenario. Taking these findings together, it seems likely that 302 optimal training in this technique would benefit from both methods of teaching - using the simulator to familiarise 303 oneself withe the procedural steps of the technique prior to performance in a cadaver and subsequently a living 304 patient.

305 Human medical and veterinary educational reviews on simulator-based training discuss how simulators 306 designed to give feedback can enhance the learning experience and facilitate self-directed learning^{6,7,30}. As such, 307 our simulator was specifically designed to provide feedback to students during performance of the procedure, by 308 allowing physical collection of a CSF sample. We hypothesized that this feature would enhance user experience 309 in the simulator group compared to the cadaver group. However, our data did not support this hypothesis - students 310 in both groups rated their experiences equally. This finding may have been confounded by the fact that, despite 311 not being able to obtain a CSF sample in the cadaver group, the session facilitator provided verbal feedback to 312 students on whether they had been successful in placing the needle into the LSS. In most cases, it was possible 313 for the session facilitator to determine if the needle was placed into the LSS. However, this can be time-consuming 314 and in a self-directed session it would be challenging for a novice user to determine if they had successfully 315 performed the procedure. In contrast, the simulator provides immediate feedback and indication of success to the 316 user. For this reason, we predict that a difference in user experience would be detected if the simulator was tested 317 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.¹⁸ Further research is required to understand the role of such simulators in self-directed learning scenarios and investigate whether skill transfer to a living patient is comparable to that following training on
 cadavers. Such investigations will guide the integration of simulators into the veterinary curriculum in the future.⁵
 This study has some limitations including the recruitment of students on a volunteer basis, which may

This study has some limitations including the recruitment of students on a volunteer basis, which may 325 have introduced volunteer bias into the study.³¹ Furthermore, while the students had not performed the CSF 326 327 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 328 have resulted in biased feedback on the simulator design. Simulator design was sufficient for the aims of this 329 study. However, certain features of the simulator would benefit from further optimisation. For example, with the 330 existing fluidic system it was difficult to maintain a consistent speed of CSF flow between users due to pressure 331 changes inside the tubing as fluid was removed with each subsequent use. The authors do not feel that this feature 332 influences the ability to effectively learn the procedural steps involved in this technique. Furthermore, the current 333 3D-printed model lacks the flexibility of a vertebral column in vivo. Integrating flexible filaments into the 334 vertebral articulations of the 3D-printed model would enhance the resilience and fidelity of the model.¹⁶ Future 335 iterations of the model would benefit from optimising these features. 336

337 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.

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431 432 **Tables**

- 433
- 434 *Table 1: Results of the expert validation survey (n=4)*

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

435 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree

436 CSF = cerebrospinal fluid, IQR = interquartile range

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Criteria	Criteria Number of students		
	Cadaver group	Simulator group	
Correct identification of the L6 spinous process	5/16	16/16	<0.0001
Successful insertion of the needle into the LSS	11/16	14/16	0.20
Successful collection of a CSF sample	0/16	14/16	<0.0001
Number of attempts			p-value ^b
Less than 3	4/11	6/14	>0.99
3 or more	7/11	8/14	-

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Table 3: Student performance as recorded via the self-assessment survey (n = 16 per group)

^a Chi-squared test ^b Fisher's exact test. Significant p-values are highlighted in bold.

CSF = cerebrospinal fluid; LSS = lumbar subarachnoid space.

	Cadaver		Simulator			
Question	Median	IQR	Median	IQR	p-value ^a	
I was able to position the cadaver/model easily for CSF collection	5	5-5	5	5-5	p = 0.30	
I was able to palpate the anatomical landmarks	4	4-5	4	4-5	p = 0.85	
I was able to identify the location for needle insertion	4	4-5	4	4-5	p = 0.98	
I was able to insert the needle through the skin and muscle easily	5	5-5	5	4-5	p = 0.23	
I was able to determine when the needle was in the correct location to collect CSF	4	3.25-4.75	4	3.25-5	p = 0.82	

447 ^a Mann-Whitney test

448 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree 449

CSF = cerebrospinal fluid, IQR = interquartile range

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

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

453 ^a Mann-Whitney test

454 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree

- 455 CSF = cerebrospinal fluid, IQR = interquartile range
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457 **Figure captions** 458

- 459 Figure 1: Flow chart of the study design
- 460 CSF = cerebrospinal fluid, LSS = lumbar subarachnoid space
- 461
- 462 Figure 2: Preparation of CT images for 3D-printing
- 463 A + B, Volume rendered 3D reconstruction of the lumbosacral vertebral column of a healthy dog.
- 464 C, Digital model, amended in order to correct artefacts to ensure integrity of anatomical landmarks, and ensure 465 patency of the vertebral canal and L5/L6 foramina during the printing process.
- 466 D, Slight flexion applied to L3-L5 portion of the digital model to simulate the flexed position of the pelvic limbs 467 during CSF sampling.
- 468

469 *Figure 3: Constructing the simulator using the 3D-printed model*

- 470 A, 3D-printed model of the lumbosacral vertebral column.
- 471 B, Insertion of latex tubing into the vertebral canal to facilitate flow of 'cerebrospinal fluid'.

- 472 C, Addition of ethylene-vinyl acetate (EVA) foam to represent the ligamentum flavum.
- 473 D, Plastic mould used to house the 3D-printed model during the addition and solidification of the ballistic gel.
- 474 E, 3D-printed model embedded in 15% ballistic gel following 24-hours refrigeration, still inside in the plastic
 475 mould.
- 476 F, Final model consisting of the 3D-printed model, ligamentum flavum (EVA foam), and soft tissue (ballistic gel)477 and tubing to facilitate CSF flow.
- 478 Figure 4: Completed construction of the simulator
- 479 A, The final model (Figure 3F) is inserted inside a life-sized soft toy dog. Synthetic skin is placed at the
- 480 appropriate level for CSF sampling at the LSS. A 1-litre bag of saline is attached to one end of the latex tubing
- 481 via an administration set. The latex tubing is primed with saline. The other end of the latex tubing is clamped with
- 482 artery forceps. An infusion pressure bag ensures a constant pressure within the latex tubing to allow flow of CSF.
- 483 B, Successful collection of CSF at the LSS using the simulator.