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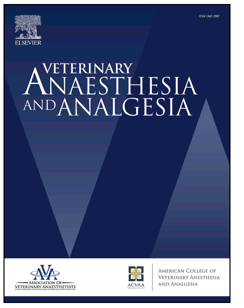

Development and evaluation of an anatomically designed and 3D printed device to enhance orotracheal intubation success in rabbits by inexperienced veterinarians

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RESEARCH PAPER

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Development and evaluation of an anatomically designed and 3D printed device to enhance orotracheal intubation success in rabbits by inexperienced veterinarians

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Abstract

Objective To assess whether the use of a 3-dimensional (3D) printed device enhances the success rate of orotracheal intubation in rabbits.

Study design Prospective, crossover randomized controlled trial.

Animals A total of six mixed-breed rabbits.

Methods A device to guide the endotracheal tube was designed based on computed tomography images and then manufactured using 3D printing. Rabbits were randomly assigned for intubation by two inexperienced veterinarians using the blind (BLI), borescope- (BOR) or device- (DEV) guided techniques. Success rate, number of attempts, time to success, injury scores and propofol dose were recorded and compared. Significance was considered when $p < 0.05$.

Results Success rate was higher in DEV (58.3%) than in BLI (8.3%) ($p < 0.023$), but not different from BOR (41.6%). Total time until successful intubation was lower in DEV (45 ± 23 seconds) and BOR (85 ± 62 seconds) than in BLI (290 seconds; $p < 0.006$). Time for the successful attempt was lower for DEV (35 ± 10 seconds) and BOR (74 ± 43 seconds) than in BLI (290 seconds; $p < 0.0001$). The propofol dose required was lower for DEV (2.3 ± 1.2 mg kg^{-1}) than for BLI (3.4 ± 1.6 mg kg^{-1}) ($p < 0.031$), but not different from BOR (2.4 ± 0.9 mg kg^{-1}). Number of attempts and oxygen desaturation events were not different among techniques ($p < 0.051$ and $p < 0.326$, respectively). Injury scores [median (range)] before and after attempts were different in BLI [0 *versus* 1(0–3), $p < 0.005$] and BOR [0 (0–1) *versus* 1(0–3), $p < 0.002$] but not in DEV [0 (0–2) *versus* 0 (0–3), $p < 0.109$].

Conclusions and clinical relevance The device facilitated orotracheal intubation with a time similar to the borescope-guided technique but faster than the traditional blind technique.

Keywords anesthesia, airway, exotic, lagomorphs, mortality, ventilation.

Introduction

Orotracheal intubation is considered a challenging maneuver in rabbits because of the species unique anatomical characteristics (Flecknell 2006). Complicating features include large incisors, a narrow oral cavity, a broad tongue, limited mouth opening, an acute angle between the mouth and the larynx and a small glottis prone to laryngospasm (Varga 2017). Unfortunately, masks and supraglottic devices do not provide a completely secure airway. Facemasks allow leakage of volatile anesthetics, increase equipment dead space and prevent efficient positive pressure ventilation (Bateman et al. 2005; Wenger et al. 2017). Species-specific supraglottic devices may compress the larynx or may invade the laryngeal vestibule creating a possible respiratory obstruction (Engbers et al. 2016; Wenger et al. 2017). It has been speculated that the difficulty in airway control contributes to the high mortality rate in rabbits during anesthesia (Brodbelt et al. 2008).

Several techniques have been investigated to improve success of orotracheal intubation. The blind technique for intubation in rabbits is the traditional technique (Alexander & Clark 1980; Fick & Schalm 1987; Kruger et al. 1994). Other techniques involve nasotracheal intubation (Stephens Devalle 2009), retrograde orotracheal (Corleta et al. 1992), capnography-guided (Lee et al. 2019), polypropylene catheter-guided intubation (Thompson et al. 2017) and endoscopic-guided intubation (Comolli et al. 2020; Freitag et al. 2020). Among these, intubation using blind and borescope-guided (a rigid or flexible instrument used to inspect the inside of objects through a small hole) techniques are some of the most used alternatives. However, both require skill and practice (Saldanha et al. 2020).

With the use of medical imaging techniques, such as computed tomography (CT) scans, in combination with 3-dimensional (3D) design and print, a wide spectrum of innovative medical devices can be developed (Aimar et al. 2019; Oberoi et al. 2020; Clausse et al. 2020). The current research aimed to develop and evaluate a 3D printed device designed to facilitate orotracheal intubation in rabbits. We hypothesized that orotracheal intubation guided by a 3D printed device designed from an airway CT scan will enable inexperienced veterinarians to intubate rabbits at a higher success rate and in a shorter time compared with blind or endoscope-assisted techniques.

Materials and methods

The study was conducted after approval by the ethics committee of the Faculty of Veterinary Sciences, UNCPBA, Argentina (Act 087/02).

Experimental design

The study was performed in two stages: stage I involved device design and development, and stage II entailed the evaluation of device effect during orotracheal intubation in anesthetized rabbits. In stage I, a clinically healthy male New Zealand rabbit, aged 6 months and weighing 3.4 kg, provided by the research colony of the Faculty of Veterinary Sciences (UNCPBA) was anesthetized with xylazine (2 mg kg^{-1}; Xilacina 2%; Laboratorios Allignani Hnos SRL, AR, Argentina) and ketamine (30 mg kg^{-1}; Ketamine 100; Chinfield SA, BA, Argentina) intramuscularly (IM) and then subjected to CT using a slice thickness of 1.2 mm and a total of 200 slices (LightSpeed ultra GE Medical Systems Device; General Electric Company, MA, USA) to scan the airway conformation with the animal in sternal recumbency and the head elevated to about 90º to the spine. Pulse rate and peripheral oxygen saturation of hemoglobin (SpO_2) were monitored using an oximeter with the probe at the base of the tail (Edan H100; Edan Instruments Inc., GD, China) during the procedure. From the images obtained, a device based on the airway conformation from the mouth to the entrance to the larynx was designed and then printed in thermoplastic polyurethane (Flex TPU; Printalot, BA, Argentina) using a Priusa i3 Steel RepRap printer system (Prusa Research as, Czech

Republic). The designed device works as a channel using an inverted ‘U’ shape, copying the width, length and angulation of the rabbit oropharynx. At the rostral end, a handle was incorporated to operate the device while avoiding the prominent incisor teeth (Fig. 1). The estimated cost of printing the device was about 2 USD.

In stage II, three orotracheal intubation techniques were compared: blind intubation (technique BLI), using a flexible borescope (technique BOR) and the 3D printed device (technique DEV). The six rabbits were healthy adult mixed-breed rabbits (three female and three male) with similar body conformation, aged 1–3 years and weighing 3.5 ± 0.2 kg, allocated to a randomized crossover design. An *a priori* performed power analysis using a confidence level of 95%, a power of 80% and an effect size of 1.1 revealed that 9 repetitions would be necessary per technique to detect relevant differences in the number of attempts among techniques. The effect size was based on the results of a pilot study with the assumption that there would be a mean difference of 2 attempts with a standard deviation of 1.8 (G*Power Version 3.1; University Düsseldorf, Germany). Therefore, 12 repetitions were chosen for each technique to compensate for possible technical issues during data collection. Animals were obtained from the research colony of the Faculty of Veterinary Sciences (UNCPBA) and housed in an environmentally controlled animal facility (Experimental facility, CIVETAN-FCV-UNCPBA). All rabbits were fed a commercial standard diet nutritionally formulated for rabbits (Cooperativa agropecuaria de Tandil ltda, BA, Argentina) and had free access to water. To reduce stress, all animals were acclimatized for 1 week before the start of the study. A veterinarian performed daily clinical monitoring of behavioural and physiologic variables during the whole study.

The rabbits, numbered from one to six, were divided into two groups of three animals each and assigned to two veterinarians (V1 and V2) that had no prior experience in rabbit intubation. The orotracheal intubation techniques were assigned to each rabbit in each group.

The assignment of numbers to each animal, animals to each group, group to each veterinarian and orotracheal intubation technique to each rabbit was performed randomly using Excel (Microsoft Corporation, WA, USA). After the three techniques were performed on each rabbit, the groups and veterinarians were interchanged and the sequence was repeated. Each rabbit underwent a total of six anesthetic events (two for each technique) with intervals of 7 days between techniques to recover from potential intubation-related injuries, stress and drugs effects.

Anesthetic protocol

Rabbits were sedated with midazolam (2 mg kg^{-1}; Fada Midazolam, Laboratorio Fada-Pharma, BA, Argentina) and xylazine (3 mg kg^{-1}; Xilacina 2%, Laboratorios Allignani Hnos SRL) IM in the pelvic limb. After 10 minutes, the right marginal auricular vein was catheterized using a 24 gauge over the needle catheter (Healthcath; Harsoria Healthcare Pvt Ltd, India). Oxygenation with a facemask (4 L $minute^{-1}$) was provided for 3 minutes before anesthetic induction. For induction, propofol (Gobbifol 1%; Laboratorios Gobbi Novag SA, BA, Argentina) was administered as an intravenous infusion using a syringe pump (EN- S7P; Shenzhen Enmind Medical Equipment Co. Ltd, GD, China) at a dose of 3 mg kg^{-1} $minute^{-1}$. At 10 second intervals, anesthetic depth was assessed to identify when complete jaw relaxation and the abolition of paw withdrawal reflex were achieved. Physiologic variables including heart rate (HR) and rhythm, SpO_2 and mean arterial pressure (MAP) were monitored using a lead II electrocardiogram (ECG; Cardiotecnica MA507; Cardiotecnica SRL, BA, Argentina), an oximeter with the probe at the base of the tail (Edan H100; Edan instruments) and a high definition oscillometric pressure monitor with a pediatric cuff attached to the left pelvic limb (Vet HDO; S + B medVET GmbH, Germany), respectively. Events such as bradycardia (< 100 beats $minute^{-1}$), hypotension (MAP < 60 mmHg) or desaturation (SpO_2 < 90%) were registered throughout the procedure until the rabbit was fully awake.

Orotracheal intubation attempts and techniques

Up to four attempts at orotracheal intubation were permitted and intubation was considered a failure if all attempts were unsuccessful. An attempt was defined as inserting a 3 mm internal diameter cuffed endotracheal tube lubricated with 2% lidocaine gel (Lidocaina jalea 2%; Laboratorio Denver Pharma SA, BA, Argentina) into the rabbit's mouth until tracheal intubation was achieved or the tube removed for a subsequent attempt. If the attempt lasted more than 120 seconds or if hemoglobin desaturation (SpO_2 < 90%) was reached, the procedure was stopped and oxygen was administered by facemask for 30 seconds. At each attempt, the withdrawal reflex in response to paw digit pinch from an investigator's nails and jaw muscle tone were assessed and the propofol infusion was administered as required to maintain anesthesia. Propofol dose, number of attempts, total time required and time duration of the successful attempt at intubation were recorded. In addition, an endoscopic evaluation of the larynx with a flexible 5.5 mm borescope connected to a computer (MaxiVideo MV105; Autel Intelligent Technology Corp. Ltd, GD, China) was performed by an independent veterinarian who was not involved in the study, immediately before and after the attempts sequence for each rabbit. For each visual inspection, an injury score was assigned using a scoring system for lesions established before start of the study (Appendix A). Once the procedure was completed, a single dose of meloxicam (1 mg kg^{-1}; Meloxivet 0.5%, Laboratorios John Martin, BA, Argentina) was administered subcutaneously to each rabbit and the rabbit was returned to the group when fully awake. At the end of the study animals from both stages were returned to the research colony.

BLI technique

With the rabbit in sternal recumbency, an assistant held the head in dorsiflexion, aligning it with the neck, so that the head was elevated to approximately 90º to the thoracic spine. The mouth was opened and the tongue retracted with the help of a gauze. Prior to the intubation attempt, a 1 mm diameter stainless steel stylet of our own manufacture, with a blunted end and not protruding from the tube, was inserted into the endotracheal tube. Blind intubation was guided by breath sounds and clouding of the tube. Successful intubation was confirmed by capnography using a portable mainstream capnograph (MEMO Capnograph; Capnomed Medical Co. Ltd, JI, China).

BOR technique

The rabbit was positioned and managed as described for the BLI technique. The operator used the nondominant hand to handle the endoscope and the dominant hand to introduce the endotracheal tube. The same borescope used for the endoscopic evaluation of the larynx was used to guide the procedure (MaxiVideo MV105; Autel Intelligent Technology Corp. Ltd). Similarly to the BLI technique, a stylet was inserted in the endotracheal tube. The endotracheal tube was introduced laterally to the borescope and inserted through the glottis. The success of intubation was confirmed by direct observation and capnography.

DEV technique

The rabbit was positioned as described for the BLI technique. The device was internally and externally lubricated with 2% lidocaine gel. The operator gently introduced the device until a slight resistance to insertion was perceived. Afterwards, the endotracheal tube without the stylet was slowly introduced using the device as a guide while hearing breath sounds to confirm the correct direction. Success of intubation was confirmed by capnography.

Statistical analysis

The first evaluation of the distribution of residuals, Q-Q plots, and Shapiro–Wilk test showed that data were not normally distributed; as a result, a generalized mixed-effects model was used. Propofol dose, total time until successful intubation, time until successful attempt and number of attempts were analyzed with a gaussian family with a link function ‘log’. The rate of success and desaturation events during intubation were analyzed with a binary family with a link function ‘logit’. Initially, the techniques and operators were included as fixed effects and the individual identification number (ID) as a random effect. Injury scores differences observed before and after intubation for each technique were analyzed using a Poisson family with a link function ‘sqrt’, including the time of score measurement and operator as a fixed effects and individual ID as a random effect. Fisher’s least significant difference was used as *post hoc* test in all cases. The RStudio software Version 2022.12.0 (RStudio, MA, USA) was used for the statistical analysis. A p value < 0.05 was considered statistically significant.

Results

All rabbits completed the study without complications. There were no differences between V1 and V2 for any of the variables under study and as a result, the comparisons between techniques was based on the sum of the 12 repetitions. Orotracheal intubation success rate was significantly higher using technique DEV than technique BLI ($p = 0.023$) but not different from technique BOR (Fig. 2). Total time until successful intubation and time of the successful attempt were significantly lower in techniques DEV and BOR than in technique BLI ($p = 0.006$ and $p < 0.0001$, respectively; Table 1). The dose of propofol was significantly lower for technique DEV than technique BLI ($p = 0.031$) but not different from technique BOR. The number of attempts to successful intubation was not different among techniques (p

= 0.051; Table 1). No events of bradycardia or hypotension were recorded. $SpO_2 < 90\%$ occurred on two occasions in technique BLI, once in technique BOR and none in technique DEV ($p = 0.326$). Injury scores were significantly higher after intubation in techniques BLI ($p = 0.005$) and BOR ($p = 0.002$) (Table 2). No differences before and after intubation were detected for the DEV technique ($p = 0.109$).

Discussion

In the current study, a specific 3D printed device was evaluated as a guide for blind orotracheal intubation in rabbits. The CT scans were useful for obtaining the measurements of the rabbit airway to generate a design of a device to be used to guide insertion of an endotracheal tube. CT and magnetic resonance imaging has been previously used to design a novel biomimetic 3D printed rabbit airway model for nasotracheal intubation training (Oberoi et al. 2020).

The device generated in the current study proved to be a low cost, simple instrument and 3D printing was fast and only required a regular 3D printer machine and thermoplastic polyurethane filament. This material has rubber-like elasticity, resilience and durability and is one of the most commonly used 3D printing filaments. The file to print the device is available free of charge and can be downloaded by scanning the QR code.

The benefit of the device was evaluated and compared with the traditional blind and the borescope-guided techniques. Results of this study showed that when two veterinarians inexperienced in tracheal intubation in rabbits attempted orotracheal intubation in rabbits, the use of the device was as effective as the borescope-guided technique and superior to the traditional blind technique.

The mean success rate was expectedly low for technique BLI (8%). Similar results have been previously reported among inexperienced veterinarians (Fusco et al. 2021). By contrast, for trained veterinarians, a success rate above 77% has been documented (Saldanha et al. 2020). Despite there being no statistical differences in the present study between techniques DEV (58%) and BOR (41%), using the 3D printed device resulted in a significantly shorter time to successful intubation than the blind intubation technique and half the time to intubation using a borescope. Propofol requirements during attempts at orotracheal intubation were lower using the orotracheal device than during blind intubation, but not different from using the borescope. The difference in propofol requirement between techniques DEV and BLI can be explained by the higher success rate in technique DEV with a shorter time to intubation and reduced need for propofol. The propofol doses and total time until successful intubation were not significantly different between techniques DEV and BOR (45 *versus* 85 seconds), suggesting that a similar anesthesia plane was required for toleration of the 3D printed device and the borescope. Regarding the number of attempts until successful intubation, there was no difference between techniques BOR and DEV; however, there was only one successful intubation in technique BLI and that required 3 attempts. Despite the lack of difference in the number of attempts, injury scores were significantly higher for techniques BLI and BOR but not for DEV. Traumatic injuries during intubation in rabbits appear to be associated with contact of the endotracheal tube with the larynx (Saldhana et al. 2020). In the present study, the 3D device restricts the endotracheal tube movement near the glottis and may explain the atraumatic characteristic of the technique. An interval of 7 days was introduced between anesthetics; even so, injury was present in one rabbit in technique DEV and another in technique BOR before attempts to intubate in anesthetic episodes 2 and 3, respectively. This may indicate that 1 week is insufficient for resolution of local injury from intubation.

Potential limitations of this study have to be highlighted. First, the sample size was estimated based on the number of attempts and may have been insufficient for assessing the other outcomes. Furthermore, the lack of success of the BLI technique prevented statistical comparison, since results could be interpreted as a type II error (false negatives). Another factor that could influence the results was the improving skills of the veterinarians throughout the course of the study at intubation using blind, borescope- and device-guided techniques. With regular practice, it is probable that the time and attempts for orotracheal intubation in rabbits may be reduced further. The 3D device evaluated in the current study was designed from a rabbit weighing 3.5 kg and tested in a similar weight range of animals. It is likely that a set of 2 or 3 sizes will be necessary to cover a larger range of rabbit sizes and weights. Fortunately, the 3D design and print can be easily scaled up or down to fit different animal sizes.

Future studies comparing these techniques performed by specialized veterinary anesthesiologists and exotic animal veterinarians would help characterize the efficacy of the 3D printing device for orotracheal intubation in rabbits.

Conclusions

The use of the 3D printed device resulted in successful intubation rates and times to intubation similar to those observed after the borescope-guided technique and superior to the traditional blind technique. This 3D printed device may be an atraumatic alternative to other techniques currently used for endotracheal intubation in rabbits, particularly among inexperienced professionals.

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Authors' contributions

PN: design, execution of the study protocol, data analysis, preparation of manuscript; MC: design, data management, execution of the study protocol; FL: design, data acquisition, execution of the study protocol; ML: design, data analysis, preparation of manuscript; VC, PV and LA: design, execution of the study protocol, preparation of manuscript. MDS: design, data acquisition, execution of the study protocol, preparation of manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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Figure 1 (a) Digital model of rabbit oropharynx and trachea generated from computed tomography images (orange) with the designed device inside (blue). (b) 3-dimensional printed device obtained from the digital model. The device presents an inverted ‘U’ shape and respects the angulation of the rabbit oropharynx. At the rostral end, a handle was incorporated to operate the device while avoiding the prominent incisor teeth. The QR code opens a link to the file to print the device.

Figure 2 Mean success and failure rate (percentage) for three endotracheal intubation techniques performed by two inexperienced veterinarians: blind (BLI), borescope-guided (BOR), or 3-dimensional printed device guided (DEV) ($n = 12$) in anesthetized rabbits.

* Significantly different from technique BLI ($p = 0.023$)

Appendix A. Injury score chart of lesion severity in the oropharynx.

Score	**Description***
0	No evident alterations
1	Edema and/or hyperemia
2	Edema and/or hyperemia with ulceration
3	Edema and/or hyperemia with ulceration and bleeding

* Observed by visual inspection with a 5.5 mm flexible endoscope connected to a computer.

Table 1. Main variables obtained for orotracheal intubation in six anesthetized rabbits using different techniques: blind technique (BLI), borescope-guided technique (BOR) or 3-dimensional printed device guided technique (DEV). Data were obtained from the sum of repetitions from two inexperienced veterinarians (n = 12). Data are shown as the number (n), percentage (%), median (range) or mean ± standard deviation.

Variables	**Techniques**			***p***
	BLI	**BOR**	**DEV**	
Success rate *n* (%)	1/12 (8.3)[a]	5 /12 (41.6)[ab]	7/12 (58.3)[b]	0.023
Total time until successful intubation (seconds)	290[a]	85 ± 62[b]	45 ± 23[b]	0.006
Time for the successful attempt (seconds)	290[a]	74 ± 43[b]	35 ± 10[b]	<0.0001
Propofol administered (mg kg^{-1})	3.4 ± 1.6[a]	2.4 ± 0.9[ab]	2.3 ± 1.2[b]	0.031
Number of attempts	3	1 (1–2)	1 (1–2)	0.051
SpO_2 < 90% (*n*)	2	1	0	0.326

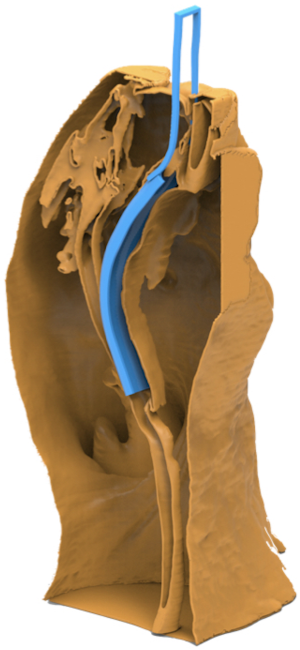
[a,b]Different superscript letters indicate significant difference among techniques ($p < 0.05$).

Table 2. Injury scores (0–3; 0 none, 3 severe) obtained before and after orotracheal intubation attempts in six anesthetized rabbits using three techniques: blind technique (BLI), borescope-guided technique (BOR) or 3-dimensional printed device-guided technique (DEV) (12 repetitions each). Data are reported as median (range).

Intubation technique	**Time points**		***p***
	Before	**After**	
BLI	0	1 (0–3)*	0.005
BOR	0 (0–1)	1 (0–3)*	0.002
DEV	0 (0–2)	0 (0–3)	0.109

*Statistically different from before orotracheal intubation ($p < 0.05$).

(a)



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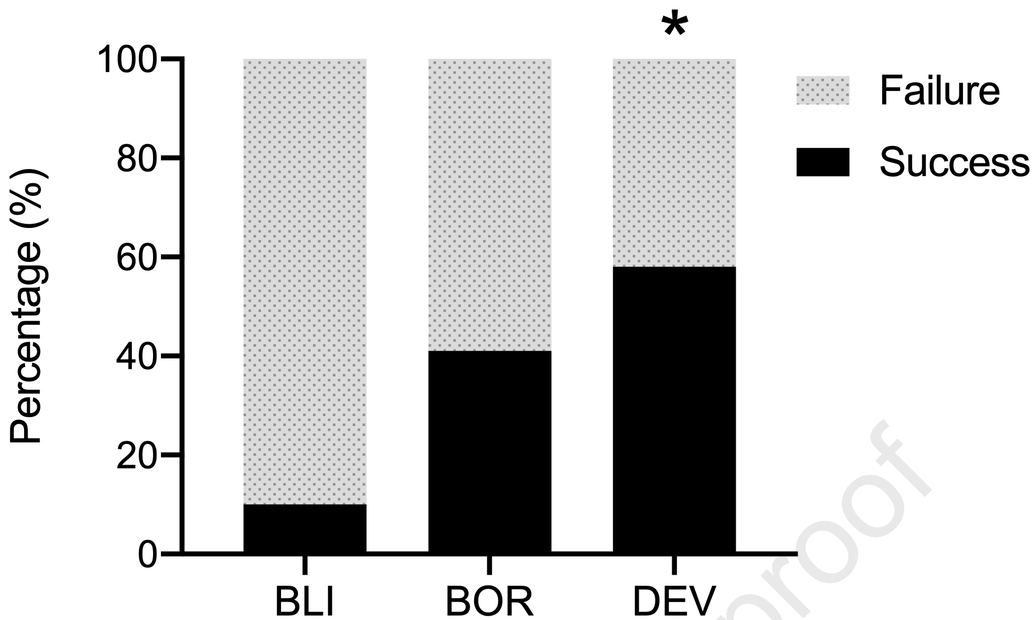
*
100
80
60
40
20
0
Percentage (%)
BLI
BOR
DEV
Failure
Success

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

None