Mini-Symposia Title: Emerging Biomedical Engineering Technologies in Veterinary Medicine

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Theme:

○ 09. Therapeutic & Diagnostic Systems and Technologies

○ 12. Translational Engineering for Healthcare Innovation and Commercialization

Mini-Symposia Synopsis

Mini-Symposia Synopsis—Max 2000 Characters

A sustainable health-care system with quality health-care services are priorities of any community worldwide. However, the rising costs of healthcare, the higher demand for services, the changes on population and global migration approaching 200 million per year represent challenges when creating a scalable and sustainable healthcare system. The past decade has brought engineering, computer and material science into medicine to decrease costs, improve services, and optimize patient care. Modern medicine includes examples ranging from robotic surgery, imaged guide procedures, 3D printing surgery, absorbable ligation systems through closed-loop target-controlled infusion systems, computerized-assisted surgery, to frameworks to support data acquisition and storage, real-time systems for physiological analyses, etc. The development of new devices, algorithms, processes, and systems are impacting a new medical field and transforming Veterinary Medicine bringing accuracy and novel therapeutic practices that follow ethical guidelines to improve research outcomes and animal welfare. This Symposium aims to present the emerging technologies in the Veterinary Medicine that are changing surgical practice, enhancing physician’s performance and improving patient care towards a sustainable healthcare system facilitating decision making, increase discovery, improving evidence-based medicine, and optimizing early intervention in critical cases. Throughout this symposium, challenges such as monitoring and image guided surgery in animals will be addressed. Monitoring animal patients during anesthesia is a vital component of providing a high standard of care in veterinary medicine. Dr. Creighton explored new technologies in monitoring, assessing patients with the use of augmented reality and alternative sensing systems. The development of models for augmented reality incorporates elements from physical systems into programmed templates. The process of modelling the physical body into code is address in this symposium by Dr. Muirhead and Dr. McSorley through the perspective and experience of an anatomist and a system engineer. Dr. Stoughton describes the process of developing a 3D printed horse brain for training nasogastric intubation minimizing the use of animals and improving performance and competency-based training. Dr. Acharya will present a novel biomaterial made from tunicates to encapsulate monitoring sensing systems for biomedicine.
Challenges and Requirements of Veterinary Medicine Design

Nadja Bressan, Faculty of Sustainable Design Engineering, University of Prince Edward Island; Catherine Creighton, Atlantic Veterinary College, University of Prince Edward Island

Abstract – The requirements to design solutions for veterinary medicine may represent an engineering challenge implying the knowledge of several different species anatomy. The aim of this short communication was to discuss the challenges of developing engineering solutions for veterinary medicine and the solutions demonstrated by this group.

I. INTRODUCTION

Veterinary Engineering is a new discipline, combining veterinary medicine and biomedical engineering. This is a logical combination, as there is significant overlap in both knowledge among researchers and the science being pursued. Often both groups are searching for new techniques or methods to improve drug delivery, analgesia or patient monitoring.

II. METHODS

Our projects range from a robotic surgery assistant device, 3D printed simulator to augment reality tools for anesthesia. When developing robotic systems for Veterinary Medicine (VM), we aim to remotely operate animals changing the practice as currently performed. However, the requirements of robotic surgical systems (RSS) such as decision-support, navigation, and object recognition when designing for veterinary medicine represent a different challenge. Decision support systems (DSS) aim to support users when making decisions while analysing pre-existing data [1]. The challenge in veterinary medicine is lack of monitor designed for veterinary use and consequently the goodness and the accuracy of the data collected. In the other hand, the navigation component of RSS is extremely challenging due to the variability on the species. The development of robotic systems shall be as flexible as the diversity of animal anatomies to navigate inside a snake or a whale. The object recognition for VM shall be able to follow the movements of the surgeon and recognize organs and tissue from different species. Our group is also developing an augmented reality tool (AR) for anesthetic blockage procedures. The main challenge of utilizing augmented reality to veterinary procedures has been to create a tool that guide the physician through the anesthetic blockage technique without utilizing a second device, such as ultrasound or nerve stimulation that fits a diverse size of animals. Another two projects encompass the design and development of heart rate monitor for reptiles and a pulse oximeter for horses. The reptiles present a few challenges, from the positioning of the heart, to its anatomy composed basically by three chamber heart [2]. The placement of any device to monitor the reptiles heart requires a deep understanding of the specie, for example chelonians would have probes placed between forelimbs and the neck, crocodilians would have the probe placed on their back, depending on animal shape, size and heart location [2]. The large animals present a different challenge when monitoring the cardiovascular system, where the placement of the measuring probe is fundamental to accurately measure the heart rate during exercises, anesthesia, surgery, and/or recovery. Recently, researchers from Standard measured for the first time the heart rate of blue whale free diving enabling the scientists to understand the dynamics of the whales’ cardiovascular system [3].

III. DISCUSSION & CONCLUSION

The aim of this short communication was to discuss the challenges of developing engineering solutions for veterinary medicine and the solutions demonstrated by this group. The fundamental requirements to design for VM can be summarized as follow: a)Anatomy/Physiology: it may sound redundant that the design of physiological monitors depends on their physiology, however that becomes a requirement when the animal has three hearts, or senses the environment through vibrations, or even use a process called cloacal respiration to breath. The anatomy of vital organs such as the heart or the lungs becomes imperative when designing a vital signal monitor; b) Instrumentation: the standard sensors utilized for decades to measure heartbeat, blood pressure, or to make an image of organ may not be useful in VM. New sensors have been developed to sense heartbeat, as fabric sensors, novel techniques applied to perform routine practices as augmented reality, etc. The instrumentation utilized to solve VM challenges is a brand-new area of research; c)Communication: the wireless feature of physiological devices is fundamental for VM, considering most of the times, the patients are large (horses) and need to recover in individual rooms, or small and active (snakes) and need to be monitored in their environment. Our group has successfully modeled a horse head for student training, a dog stifle, and equipment to perform minimally invasive surgery. In the short time that the engineers and veterinarians have been working together in the discipline of veterinary engineering, we have achieved a solid foundation upon which to build the future of this discipline.

REFERENCES

Minimally Invasive Surgery in Veterinary Medicine

Adam Ogilvie, Atlantic Veterinary College, University of Prince Edward Island

Abstract—Minimally invasive surgery (MIS) is becoming more popular in veterinary surgery. There continue to be challenges to the veterinary surgeon who performs laparoscopy and thoracoscopy. This presentation will focus on some of the challenges we have identified during clinical practice.

I. INTRODUCTION

Minimally invasive surgery (MIS) is becoming more popular in veterinary surgery. The list of commonly performed procedures continues to grow, including cholecystectomy, adrenalectomy, gastropexy, ovariectomy, organ biopsy, etc [1]. There have been concomitant increases in numbers of publications, continuing education courses, and professional societies dedicated to the discipline. MIS has been shown to result in less patient discomfort and increased post-operative mobility when compared to traditional “open” surgery. Other purported benefits include improved visualization through magnification, high-definition cameras, increased access to anatomical regions, and reduced incisional complications.

There continue to be challenges to the veterinary surgeon who performs laparoscopy and thoracoscopy [2,3]. This presentation will focus on some of the challenges we have identified during clinical practice. We will begin with an introduction to modern veterinary MIS. Common instrumentation and abdominal/thoracic access techniques will be discussed. We will use case examples to highlight the key aspects of MIS, and to identify the following challenges.

II. CHALLENGE I – PATIENT VARIABILITY

The veterinary MIS surgeon has a caseload that spans cats, toy-breed dogs, and giant breeds. Further, MIS is being used in exotics/pocket pets and farm animal species. This size discrepancy makes standardized equipment difficult to adapt to the individual patient. Instrumentation that is commonly used has been designed for human use or adapted from previous human designs. Most equipment is 5mm in diameter, and some pieces of equipment are 10 mm in diameter. These sizes may be too large for felines/pocket pets. Conversely, many organs we remove during MIS are too large to fit through a 10mm cannula, precluding the use of MIS. Finally, the length of MIS equipment is often too long for the body size seen in companion animals, reducing dexterity and efficiency of movement.

III. CHALLENGE I – PATIENT VARIABILITY

Veterinary medicine is a “fee-for-service” business. MIS capital equipment is expensive (tower, camera, telescopes, insufflation, image-capture, etc). MIS procedures may take longer to perform. Many veterinary practices bill client’s item-by-item, including anesthetic time, surgical time, equipment, drugs, etc. There are ways to reduce this economic challenge, such as “all-inclusive” billing, where the costs are distributed over many patients. These types of systems are based on a “typical” case, but still have to reflect the costs of purchase, maintenance, and inventory management for MIS equipment. Further, some MIS equipment (staplers, vessel sealing devices) are single-use only. Many companies are moving away from re-usable equipment. This tends to result in higher cost-per-unit. For clients with limited funds, these increased costs can be a significant factor in choosing traditional “open” surgeries over MIS.

IV. CHALLENGE III – DISEASE RECOGNITION

Many large referral institutions and veterinary teaching hospitals have access to an array of diagnostic tests, imaging modalities, and veterinarian who specialize in certain areas. Despite these advances, we rely on pet owners to recognize disease. Companion animal species are adept at masking disease and hiding pain. These qualities make identifying patients with mild to moderate severity of disease very challenging, and, patients may not present to a veterinary facility until their candidacy for MIS has passed.

V. DISCUSSION & CONCLUSION

As more veterinarians become familiar with MIS, these procedures are becoming more common. Veterinary engineers in conjunction with veterinary surgeons have the potential to contribute to the growth and refinement of MIS by designing and developing instrumentation that is specific for veterinary patients. By removing or reducing financial obstacles, more clients may be inclined to pursue MIS. Finally, engineering solutions to facilitate early disease recognition may increase the number of patients who are offered MIS.

REFERENCES

Biomedical Engineering for Local Nerve Blocks in Dogs

Catherine Creighton, Atlantic Veterinary College, UPEI; Tammy Muirhead, Atlantic Veterinary College, University of Prince Edward Island

Abstract—This paper discusses how Veterinary Medicine and Biomedical Engineering work together to advance the way local nerve blocks are performed, with the goal of improving patient care during canine orthopedic surgical procedures.

I. INTRODUCTION

Injury to the cranial cruciate ligament is common in dogs, resulting in significant welfare and financial impacts to dogs and their owners [1]. Several surgical repair techniques exist, and the choice of repair method is often determined by surgeon preference as well as the size of the dog [2]. Analgesia is of paramount importance during surgical repair. Analgesia is commonly provided by use of an epidural injection at the lumbosacral space using a combination of the opioid morphine and the local anesthetic bupivacaine. Drawbacks to this method include temporary paralysis of the hind limbs, urine retention and delayed hair regrowth at the site of injection [3]. A combination of femoral and sciatic nerve blocks using bupivacaine have been used to attempt to avoid these complications, and have been shown to be not worse in terms of analgesia compared to epidural injection [4,5,6]. These nerve blocks are usually performed using ultrasound guidance for nerve location, and several techniques exist for each nerve block [7]. We have used Microsoft HoloLens in conjunction with palpation of anatomic landmarks to successfully locate the femoral and sciatic nerves in seven cadaver dogs.

II. METHODS

Seven cadaver dogs, ranging in weight from 8.6 kg to 26.8 kg, were used. To locate the femoral nerve by palpation, a combination of the Campoy and the Mahler techniques was used [7]. To locate the sciatic nerve by palpation, the lateral approach technique by Campoy was used [7]. The dog legs were then dissected to determine how effective the nerve block would have been in a live dog; staining of the nerve with tissue dye indicates success of the injection technique. For the femoral nerve, a 4-6 cm skin incision (depending on the size of the cadaver) was made on the medial aspect of the thigh extending from the inguinal region toward the stifle. Blunt dissection of the underlying fascia was performed to expose the pectineus muscle, the caudal sartorius muscle and the inguinal ligament. Deeper blunt dissection was gently performed to isolate the femoral nerve between the three aforementioned structures and also identify the placement of the tissue dye injected. For the sciatic nerve, a 3-5 cm skin incision was made between the ischiatric tuberosity and the greater trochanter tracing cranially to caudally. Blunt dissection was used to identify the large sciatic nerve and the injected tissue dye. Measurements between palpable anatomic landmarks and femoral and sciatic nerves were taken on each cadaver during dissection. Measurements were also taken to create an algorithm that would allow the Microsoft HoloLens to locate the femoral and sciatic nerves without the need for palpation.

III. RESULTS

Seven cadaver dogs were used to perform femoral and sciatic nerve blocks using tissue dye. In six of seven dogs, both left and right femoral nerves were injected, for a total of 13 injections. Dissection of the femoral nerve revealed staining of the nerve in all sites. Distance from the nerve to the dye ranged from 0 mm – 20 mm, with an average distance of 3 mm, indicating a successful block. In five of seven dogs, both left and right sciatic nerves were injected, for a total of 12 injections. Dissection of the sciatic nerve revealed staining of the nerve in all sites. Distance from the nerve to the dye ranged from 0 mm – 4 mm, with an average distance of 0.3 mm, indicating a successful block. Palpation of the anatomic landmarks for injection of tissue dye in the area of the femoral and sciatic nerves resulted in dye being within 3 mm of the nerve for a length of >6mm along the nerve length in 70% of injections. Microsoft HoloLens was successfully used to create an augmented reality 3D model of the dog leg. With further development, this can be used to teach femoral and sciatic nerve blocks without the need for palpation of anatomic landmarks.

IV. DISCUSSION & CONCLUSION

Veterinary medicine and biomedical engineering have worked together to advance technology in performing local blocks in dogs. This will improve analgesia and therefore welfare of dogs undergoing surgical procedures to repair cranial cruciate ligament rupture.

REFERENCES

Mixed Reality for Veterinary Medicine
Modelling Canine Femoral Nerve

N. Wilkie, G. McSorley, Nadja Bressan, Faculty of Sustainable Design Engineering, University of Prince Edward Island

Abstract—The femoral nerve blockage is a procedure that aims to provide anesthesia to the hip, anterior thigh, and knee. This anesthetic procedure presents multiple challenges when performed in veterinary patients with diverse anatomy and physiology. A mixed reality application has been developed, including a custom 3D model of a canine’s hind leg, to guide practitioners in the femoral nerve block procedure. This study aims to validate the workflow used in developing the mixed reality application and custom 3D model, as well as to test the utility of the application in guiding an anesthesiologist in completing a femoral nerve block in the hind leg of a canine.

I. INTRODUCTION

Augmented reality is the use of technology to superimpose three dimensional models upon videos of the real world. Virtual reality blocks out the physical world and immerses a user in a digital world. Mixed reality combines these technologies to merge interactions with digital content and physical environments. Mixed reality headsets are an innovative technology capable of advancing biomedical science. This technology has important applications such as training of individuals in a virtual environment [1], guiding a surgeon through a maxillofacial surgery with 3D models from the patients CT scans [2], and displaying “medical image segmentations” [3] of CT or MRI scans. To date, however, its application to veterinary sciences has been limited. Currently, the Atlantic Veterinary College (AVC) at the University of Prince Edward Island performs an epidural block in the lower back of a canine before surgeries on their hind legs. Peripheral and regional nerve blocks are commonly used to provide analgesia to anesthetized veterinary patients. Epidural administration of morphine, an opioid, and bupivacaine, a local anesthetic, is a commonly used technique to provide intraoperative and postoperative analgesia as well as reduce intraoperative anesthetic requirements [4]. Significant side effects can be seen following epidural administration. These side effects include hypotension, temporary motor paresis, and urine retention [5].

II. METHODS

The leg of a medium sized canine cadaver was dissected to clearly reveal the femoral nerve. No ethical approval was required to perform experiments with a canine cadaver. A Canon Rebel T5i camera with the standard lens was used to capture images of the leg required for the creation of a 3D model within 3DF Zephyr. The camera was set with an aperture of f/22, an ISO of 200, and a varying exposure time dependent on the lighting in each shot. Mirror lock and a two second timer were set to avoid camera shake from the operator. Pictures were taken level with the leg in a clockwise rotation. Overlap between captured images was 80 percent, as recommended by the 3DF Zephyr user manual [6]. This procedure was repeated at varying camera heights and angles to ensure all sides of the leg were captured. Pictures were captured in the RAW format to allow for light balancing in post processing without reducing image quality.

III. RESULTS

The completed application was tested utilizing a cadaver dog. The anesthesiologist opened the application in the Microsoft HoloLens positioning the cadaver dog through resizing, moving, and rotating the holographic model until it is centered on top of the cadaver dog leg. Once the holographic model was positioned on top of the real leg, the anesthesiologist applied tissue marking dye (orange) with a syringe 3 ml of gauge 2.5, exactly where the femoral nerve was highlighted by the mixed reality headset. After the anesthetic procedure was completed, the anesthesiologist utilized a knife to open the cadaver dog leg to explore if the marking dye was placed close to the femoral nerve.

IV. DISCUSSION & CONCLUSION

An experienced veterinary anesthesiologist performed a pilot test to verify the functionality and feasibility of using a mixed reality tool to perform anesthetic procedures, as well as the accuracy of the tool. The pilot test was completed on location at the AVC. In the test, the application demonstrated limitations in the femoral nerve location accuracy when positioning and resizing the model superimposed on the real leg. These limitations may be explained by anatomical inter-individual variability in the canine species; the leg of a Chihuahua cannot be resized to a Great Dane. The future model shall incorporate this anatomical inter-individual variability of the species as well as the classification of the nerve by leg, breed, and age of the animal.

REFERENCES

**Wet Adhesive Hydrogel for Replacing Damage Meniscus**

Gabrielle Gray and Bishnu Acharya, Faculty of Sustainable Design Engineering, University of Prince Edward Island; Adam Ogilvie, Atlantic Veterinary College, University of Prince Edward Island

Abstract—In recent years there has been increasing interest in the use of sustainable and natural materials in the preparation of advanced composites. Contrarily, there has been limited success in creating conductive hydrogels with outstanding self-healing and mechanical properties. Herein, we report advanced hydrogels which display strain-dependent conductivity, high transparency, wet and dry adhesive behavior, and exceptional mechanical performance.

I. INTRODUCTION

Canine stifle joints contain two meniscuses - the lateral and medial. They are C-shaped fibro cartilaginous disks found between the femur and tibia [1]. Hydrogels have the capability to replace damaged meniscuses associated with acute or chronic cruciate disease. In cases where a canine is suffering from cruciate disease damage is caused to the medial meniscus in 96% to 100% of cases [1]. Currently, the damaged or diseased cranial cruciate ligament can be repaired but meniscus injuries rarely heal due to the sparse blood supply so a partial or full meniscectomy is required. The meniscuses provided a load-transferring function within the stifle joint by distributing the compressive load delivered during weight bearing [2]. A meniscectomy leads to osteoarthritis and a perpetual cycle of articular cartilage degeneration [2]. Menisci replacements made of tannic acid coated tunicate-based cellulose hydrogels potentially function as a load-transferring device reducing articular cartilage degeneration and osteoarthritis associated with partial or complete meniscectomy. A hydrogel replacement will also aid in stifle joint stability by improving incongruity between the femur and tibia.

II. METHODS - COATING TUNICATE-BASED NANOCRYSTALLINE CELLULOSE IN TANNIC ACID

In a one-pot water-based process the tunicate-based nanocrystalline cellulose (NCC) was coated in tannic acid for obtaining symbols. Use a long dash rather than a hyphen for a TA@NCC suspension with a concentration of 1.2 wt%.

III. METHODS – PREPARING MENISCUS HYDROGELS

Molds of the lateral and medial meniscus’ were removed from a canine cadaver and molded using Durabond 90. These molds were then scanned using HD100 structured light scanner creating a digital image file of the molds. Unsinging the digitized meniscus files a negative was created. The negative was used to create casting molds in which the hydrogel solution was cast into. The mold has an inlet and outlet allowing solution to be injected until the casting cavity is completely filled without the presence of air. The hydrogel mixture was created using a two-step process. In the first step APS initiator, MBA chemical cross-linker and AA monomer were dissolved in 96mL of distilled water. In the next step, the TA@NCC suspension was added and magnetically stirred at a vigorous rate for 10 minutes. The mixture was then placed into a metal cage in a water bath in which it was sonicated for 10 minutes. The mixture was then placed directly into its molds and then heated at 60°C for 2 hours. Once they are removed from the molds, the hydrogels were placed in distilled water for two days. After the gels were stored for 48 hours, they were cut into one-inch sections. Theses section were placed into 50mL of AlCl3 for 2 hours before being stored in 50mL of distilled water.

IV. METHODS – COMPRESSION TESTING

Using a load cell, the femur end will be attached to the stationary top position while the tibia end is attached to the bottom position which will apply force compression the hydrogel meniscuses. A holding mechanism will be used between the bones to ensure that the lateral and medial hydrogel meniscuses do not slide out of place when the compressing force is applied. The compression data will be collected.

V. RESULTS

Using the load cell at the Faculty of Sustainable Design Engineering, the tensile properties of the hydrogels were tested. A double clamp system was used to hold the rectangular hydrogels in place. Using the LabView scrip, the load cell could begin to move in the downward direction, stretching the hydrogels. The data was collected and displayed in pound-force and displacement, which was converted to stress and strain to compare. The modulus was found giving the linear relationship between stress and strain.

VI. DISCUSSION & CONCLUSION

Using comparative testing, a better understanding of the hydrogels at varying TA@NCC percentages has been analyzed. The hydrogels produced were highly transparent, anti-inflammatory, and residue-free. The results also demonstrated that, as the amount of TA@TNCC increased the percent elongation and average adhesive force increase, while the stiffness decreased.

REFERENCES


Development of an equine nasogastric intubation simulator as an aid or alternative to the use of animals in teaching

W. Ben Stoughton, Atlantic Veterinary College, University of Prince Edward, Eagan Boire and Nadja Bressan, Faculty of Sustainable Design Engineering, University of Prince Edward Island

Abstract— Veterinary student opportunities to practice technical skills are limited by animal welfare concerns, safety and teaching animal availability. Development of an equine nasogastric intubation simulator has yet to be successfully created for use in veterinary teaching curriculum. The study aims to develop a novel equine simulator for nasogastric intubation.

I. INTRODUCTION

Colic remains one of the most common clinical presentations in equine emergency practice [1]. Nasogastric intubation is frequently required for diagnostic and therapeutic purposes. If an intestinal obstruction is suspected, the horse may need to have the stomach emptied or require oral fluids and laxatives. Consequently, being able to pass a nasogastric tube is an essential, day one skill for equine practitioners. One of the complications of poor nasogastric placement is epistaxis (nosebleed), which is caused by trauma to the ethmoids [2]. Unfortunately, veterinary student opportunities to practice this skill are often limited by equine welfare and safety limitations [3]. The study aims to develop a novel equine simulator for nasogastric intubation.

II. METHODS

Computed tomography (CT) images from a 7-year-old Newfoundland pony were used to design the 3D printed model. While costly software solutions which can convert DICOM CT data into printable 3D models exist, a low-cost manual approach was required. Solidworks is a software package created by Dassault Systems for all manner of 3D CAD design and simulation work [4]. Using Solidworks, the goal was to convert DICOM CT images to a 3D model which could be printed using an additive manufacturing process. The method used to create this model involved taking the individual images from the DICOM directory file to overlay them on planes in a solid body within the program. With these overlays in place, the areas of the CT which were voids in the structure could be traced, as showed in figure 1. The CT used for this exercise had a slice thickness resolution of 3mm, this translated into planes which were spaced 3mm apart. This model consisted of 99 planes as the length of the relevant anatomy was approximately 300mm in length, as showed in figure 2. Solidworks does have an automated trace feature built into the software package which can be used to automatically generate line profiles on high contrast images. This function was not used in this case for two main reasons. The passages of interest for this model had to be manually distinguished from the rest of the anatomy present in the CT image which could not be achieved by the auto-trace function. The accuracy of the auto-trace was inconsistent due to the contrast of the images, corrections to theses traces added 63% on average to the total time to trace one slice.

III. RESULTS

The result is a solid body with voids within which are accurate to this CT image, illustrated at figure 2. The resolution of 3mm per slice makes the transition from slice to slice abrupt. Once the negative of this solid body is printed these imperfections can be manually smoothed using abrasive material. This smoothed profile can then be used to create a silicone model using the plastic 3D printed negative as a mold. After the model is printed vibration and pressure sensors will be incorporated along the nasal passages and the oropharynx. Use of a supervisory control and data acquisition (SCADA) system will allow immediate auditory and visual feedback on correct placement of the tube and avoidance of the ethmoids

IV. DISCUSSION & CONCLUSION

This study will provide for the development of an equine simulator for nasogastric intubation. We hypothesize that this simulator will improve the ability of veterinary students to safely and successfully perform equine nasogastric intubation. By better preparing and refining the student’s skills prior to the live-animal labs, we can minimize potential pain and distress caused by poorly performed nasogastric intubation. Finally, we could directly replace or reduce the use of animals in teaching.

REFERENCES