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Evaluation of transanal minimally invasive surgery for submucosal rectal resection in cadaveric canine specimens

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Abstract

Objective: To evaluate the feasibility of transanal minimally invasive surgery (TAMIS) for submucosal rectal resection in large breed dogs.

Study design: Cadaveric study.

Sample population: Canine cadavers (n = 6) weighing between 37.5 and 60 kg.

Methods: Dogs were positioned in sternal recumbency. After rectal cleansing, a transanal access platform was placed in the rectum, and a pneumorectum was established. An area of ventral rectal wall approximately 2 × 2 cm was resected in a submucosal plane by using laparoscopic instruments and submitted for histopathological evaluation. The rectal wall defect was closed with a single-layer continuous suture pattern with barbed suture. Postoperatively, the rectum was removed en bloc and evaluated for suture or surgical penetration of the serosal surface.

Results: Submucosal rectal resection was successfully completed by using TAMIS in all dogs. The median length of resected specimens after fixation was 24.5 mm (range 9.8-26.5). In two of six dogs, suture was macroscopically visible on the serosal surface, but no dogs had evidence of iatrogenic full-thickness surgical penetration of the rectum. The median distance from the aborad extent of the suture closure line to the anocutaneous junction was 35 mm (range, 35-105).

Conclusion: Submucosal resection of the canine rectal wall was feasible in large breed dogs by using TAMIS. No evidence of full-thickness penetration of the rectal wall was seen in these cadaveric specimens.

Clinical significance: Transanal minimally invasive surgery may provide an alternative minimally invasive approach for resection for benign adenomatous rectal polyps in large breed dogs that might otherwise require a rectal pull-through.

1 | INTRODUCTION

Rectal tumors are uncommonly encountered in small animal surgical practice but can be challenging to treat depending on their type, location, and extent.[1-9] Epithelial tumors are the most common type of rectal tumor in dogs. These lesions undergo a well-known progression from adenomatous polyp to invasive adenocarcinoma as malignant transformation and invasion into deeper layers of the rectal wall occurs.[1-3] Benign neglect of these tumors is, therefore, not recommended.

The surgical approach to rectal lesions is largely dependent on the location and extent of the mass. A variety of surgical approaches have been used for management of colorectal tumors, including mucosal eversion,[4] dorsal perineal approach,[5] rectal pull-through techniques,[6,7] pelvic osteotomy via celiotomy,[8,9] and endoscopic mucosal resection with snare polypectomy.[10] While mucosal eversion and dorsal perineal approaches have been used to gain access to the distal to midrectum,[4,5] for resections of lesions located in the midrectum or proximal rectum, more invasive approaches such as rectal pull-through[6,7] or pelvic osteotomy techniques are usually required.[8,9] A recent report summarized complications associated with rectal pull-through, including fecal incontinence (transient in 45% dogs and permanent in 54% dogs), diarrhea (permanent in 25% dogs), strictures (25%), and incisional dehiscence (8%).[7]

Similar complications are reported in humans with rectal neoplasia, and, in an effort to reduce this level of morbidity, alternatives to more invasive techniques have been performed with success in clinical patients. The first generation of minimally invasive transanal approaches was termed transendoscopic microsurgery (TEM).[11] Advantages were found compared with traditional transanal excision techniques, including higher proportions of clear surgical margins and less frequent recurrence compared with transanal approaches.[11] However, the procedure required a very expensive purpose-built surgical access platform and specialized instruments.[12] It was also limited by restrictive working space and had a steep learning curve. In 2010, transanal minimally invasive surgery (TAMIS) was introduced and has been adopted more widely in the human colorectal surgery community because of reduced costs and the ability to use traditional laparoscopic instruments already available in most centers.[13] The TAMIS platform also allowed greater maneuverability of instrumentation compared with the TEM platform. In many centers, TAMIS has now become the standard of care for most endoscopically unresectable polyps and early stage (T1) rectal cancers.[14-16]

This proof of concept study was designed to evaluate whether the TAMIS methodology can be applied to large breed dogs. The objective for the study was to evaluate whether a commercially available transanal device could be used to perform TAMIS for submucosal rectal resection in large breed dogs and whether TAMIS could be performed without obvious evidence of penetration into the peritoneal cavity.

2 | MATERIALS AND METHODS

2.1 | Animals

Six large breed canine cadavers were acquired from a commercial vendor (Skulls Unlimited, Oklahoma City, Oklahoma). All dogs were shipped frozen and then transferred to a freezer at the institution where the study was performed. Cadavers were thawed at room temperature prior to use for a median of 87 hours (range 50-102).

2.2 | Bowel preparation

Digital removal of as much of the fecal material in the rectum as possible was performed preoperatively. Several tap water enemas were then performed with a surgical lavage syringe to attempt to thoroughly empty the rectum of remaining fecal debris. The indwelling lavage solution was allowed to flow from the rectum, and lap sponges were subsequently used intrarectally to absorb remaining lavage fluid. One or two 4-in square surgical sponges were then inserted deep into the rectum to form a barrier to additional fecal contamination orad to the intended site of rectal wall resection and to aid in creating a pneumorectum.

2.3 | Surgical procedure

Dogs were positioned in sternal recumbency on a perineal stand for initiation of the surgical procedure. The tail was tied cranially to facilitate rectal access. After bowel cleansing, the 4 × 4-cm GelPOINT path transanal access platform (GP-TAP; CNB10; Applied Medical, Rancho Santa Margarita, California) was placed into the rectum. The access sheath supplied with the device was initially folded inward to make it smaller and facilitate its insertion into the rectum (Figure 1A). When it was required, the supplied dilator was used to slowly dilate the rectum until the access sheath was fully open (Figure 1B). After full dilation, the access sheath was sutured to the perirectal skin with 2-0 nylon suture to prevent the

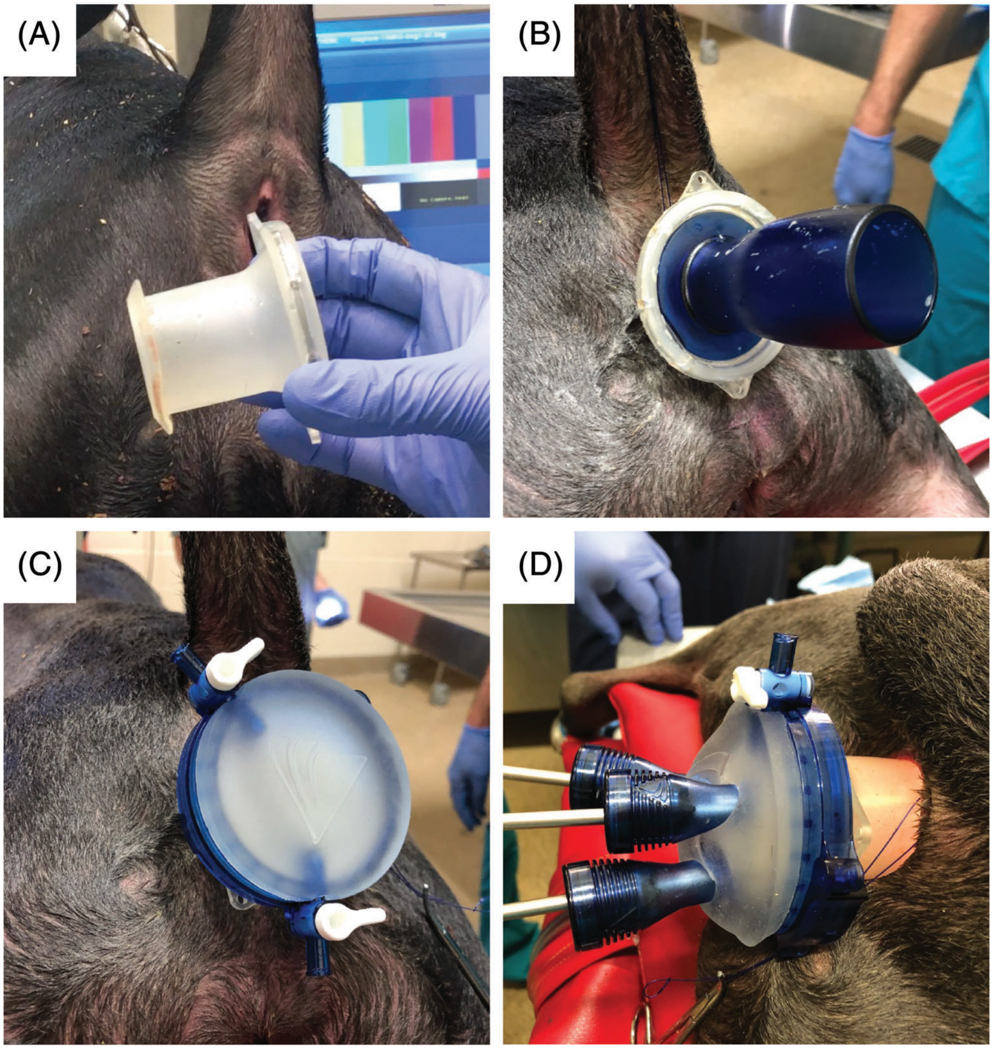

FIGURE 1 Images illustrating the placement technique for the GelPOINT path transanal access platform. A, First, the internal sheath was placed into the rectum. For placement, the end of the sheath was collapsed inward to allow initial passage. B, After sheath placement, the supplied dilator was placed to dilate the sheath after it was in position in the rectum. C, The dilator was then removed, and the GelSeal cap could be placed onto the sheath. The authors found that, in most cases, preplacement of the cannulae through the GelSeal cap prior to GelSeal cap attachment to the inner sheath was easier. D, After the transanal access platform was in position, camera and instruments could be placed through the cannulae after induction of a pneumorectum. Note how the GelSeal cap billows out and has a concave appearance after initiation of the pneumorectum

sheath from being pushed out of the rectum. After the access sheath was secured in place, the GelSeal cap was secured to the access sheath (Figure 1C). Three custom cannulae provided in the GP-TAP were then preplaced through the GelSeal cap because it was found that it was easier to preplace them rather than placing the cannulae after the GelSeal cap had been positioned on the access sheath. The GelSeal cap was then positioned onto the access sheath and secured in place (Figure 1D). A pneumorectum was initiated by using a mechanical insufflator (Karl Storz Veterinary Endoscopy, Goleta, California) to a pressure of 8 to 10 mm Hg. An area of the ventral rectal wall approximately 2 × 2 cm was selected, and the resection site was planned out by placement of small monopolar electrosurgical score marks on the rectal mucosa. Laparoscopic scissors and laparoscopic Kelly forceps were then used to perform what the operating surgeons anticipated to be a submucosal resection of the indicated area of rectal wall (Figure 2A). Resected tissue from each dog was placed in formalin and sent for histopathological evaluation to establish the depth of resection. Any iatrogenic damage to the rectal wall or obvious evidence of rectal perforation or leakage of insufflation gas was assessed at the time of surgery. A single strand of barbed suture (3-0, 6-in V-Loc180, on a CV-23 needle; Medtronic, Salem, Massachusetts) was deposited in through the access sheath, and laparoscopic needle holders (KOH macro needle holder, Karl Storz) were used to suture the defect closed with a simple continuous pattern (Figure 2B). Suturing proceeded from the most orad point of the resection in an aborad direction, and the continuous suture line was completed by taking an extra two bites of rectal wall beyond closure of the defect perpendicular to the preceding bites (Figure 2C). All intracorporeal suturing was performed by one of two board-certified surgeons (P.D.M, I.M.B) experienced in intracorporeal suturing techniques. After completion of the closure, gauze sponges were removed, pneumorectum was evacuated, and the GP-TAP was removed from the rectum. Total surgical time (in minutes) as well as the time from the start of the resection to the end of the resection and the time from the

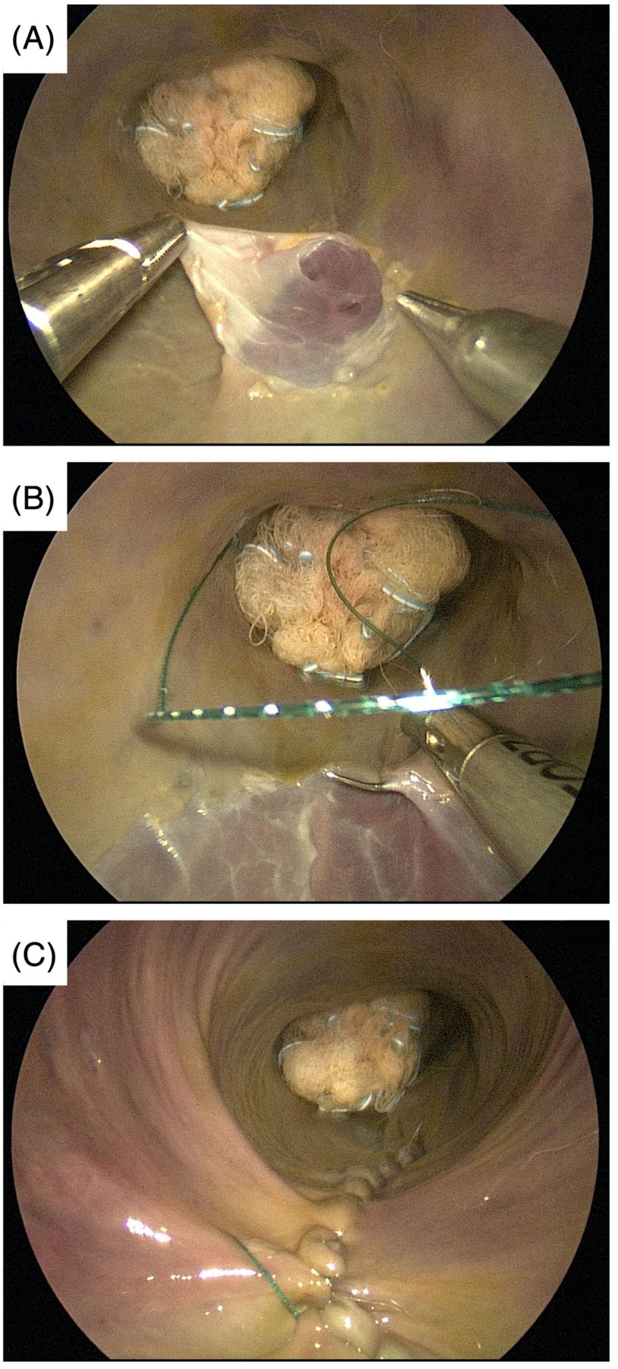

FIGURE 2 A,B,C, The stages of submucosal rectal resection with the TAMIS technique. Laparoscopic instruments were initially used to incise and dissect in the plane between the submucosa and muscular layers of the rectal wall (A). Orad, surgical sponges can be seen occluding the rectal lumen to minimize fecal contamination of the surgical site. After the rectal wall specimen had been resected and removed, barbed suture was used to initiate closure of the defect by using laparoscopic needle holders (B). The completed suture line used for closure of the rectal wall defect can be seen at the end of the procedure (C). The surgical sponges were then removed

start of the intracorporeal suture closure to its termination were recorded.

2.4 | Ease-of-placement score

A subjective score was assigned to describe the ease with which the GP-TAP could be placed in the dogs' rectums. The score was assigned subjectively by the authors by using a modified 5-point Likert scale[17] (1 = very easy; 2 = easy; 3 = medium; 4 = difficult; 5 = impossible).

2.5 | Assessment of suture penetration of the rectal wall

After completion of the surgical procedure, the rectum was removed en bloc by using a modified rectal pull-through approach. An incision was made close to the anocutaneous junction and dissection in an orad direction was performed inside the external anal sphincter muscle. Dissection was continued in the plane outside the rectal wall as far orad as possible. The sutures from the mucosal closure were manually palpated, and dissection was continued until the rectal wall could be harvested at least 3 cm orad to the most orad aspect of the suture line. After removal, the rectal wall was carefully evaluated for evidence of any full-thickness wall penetration by suture. If any sutures were visible externally (thus providing evidence of full thickness rectal penetration with suture), the presence and number of suture bites that were visible were documented.

2.6 | Assessment of peritoneal penetration, leakage, and resection location

After resection, the orad and aborad ends of the resected portion of rectum were sealed by cross-clamping with Carmalt forceps. Methylene blue was infused into tap water to a point at which blue discoloration of the water occurred. The methylene blue-water mixture was slowly infused via a 20 to 22-gauge needle into the rectal segment until it was mildly turgid to evaluate for any sign of leakage. When any leakage of the methylene blue-saline mixture was detected, it was recorded. Thereafter, the dorsal aspect of the resected rectum was incised to allow careful evaluation of the mucosal suture line (Figure 3). Evidence of defects in the mucosal closure were recorded, and measurements of the length of the suture line as well as the distance from the anocutaneous junction to the

FIGURE 3 The suture line from each specimen was closely inspected from the mucosal side after removal. Distance from the aborad end of the suture line to the anocutaneous junction is indicated by the arrows, and the length of the suture line is indicated by the dotted line

aborad aspect of the suture closure line were recorded to document the location of the resection site (Figure 3).

2.7 | Histology of resected rectum

Samples of rectal wall that were resected were submitted for histologic evaluation. They were immersed in 10% neutral, buffered formalin and fixed for at least 48 hours. Replicate sections of each sample were placed in a single cassette. The number of sections varied from four to seven, depending on the size of the sample. The sections were dehydrated through graded alcohol and xylene, then embedded in paraffin. Five-μm-thick sections were mounted on glass slides, rehydrated, and stained with hematoxylin and eosin. All slides were evaluated by the same pathologist (M.K.K.). They were digitized by using an Olympus (Tokyo, Japan) VS120 scanner, and measurements of the digitized images were made in the VS120 software. The length of each section was determined by measuring a line corresponding to the contour of the muscularis mucosa.

3 | RESULTS

3.1 | Cadavers

Six canine cadavers were acquired from a commercial source (Table 1). Dogs weighed a median of 44.3 kg (range, 37.5-60). Breeds included one each of Dobermann pinscher, Great Dane, Labrador retriever, mixed breed, Rottweiler, and Saint Bernard. Four were female and two were male castrated. The ages of the dogs could not be ascertained but were estimated to be in the range of 3 to 5 years (three dogs) and 5 to 7 years (three dogs).

3.2 | Surgical procedures

The GP-TAP were placed successfully in all dogs. The ease-of-placement score was 1 out of 5 in three dogs and 2 out of 5 in three dogs. In one dog (weight = 37.5 kg), a persistent band of ventral rectum protruded from the inner ring of the device (Figure 4). The approximate sizes of the largest dimensions of the fresh resected rectum immediately after resection were 2 × 2 cm in three dogs, 2 × 2.5 cm in one dog, 2 × 3 cm in one dog, and 2.8 × 3.2 cm in one dog. In each case, only one pack of barbed suture was used. In three dogs, eight suture bites were required to close the defect; in two dogs, nine suture bites were required; and, in one dog, the number of suture bites required to close the defect was not recorded. Complications were identified in only one dog in which a small amount of iatrogenic damage to a band of ventral rectal wall that protruded from the inner ring occurred. The median total surgical time was 31.5 minutes (range, 21-53). The median time taken to complete the resection was 15 minutes (range, 6-28). The median time taken to suture the defects was 17 minutes (range, 12-25).

3.3 | Assessment of suture penetration of the rectal wall

After resection of the rectal wall in four dogs, there was no evidence of full thickness penetration of the rectal wall by any sutures. In one dog, there was evidence of one suture bite visible from the serosal side, and in one dog there was evidence of two suture bites visible from the serosal side (Figure 5).

TABLE 1 Summary data from six canine cadavers that underwent TAMIS for submucosal resection of the rectal wall

Dog No.	Weight, kg	Breed	Maximum length of resected rectal wall after fixation, mm	Length of suture line, mm	Distance—anocutaneous junction to aborad margin of suture line, mm
1	60	Rottweiler	24.8	30	35
2	41.5	Labrador retriever	19.3	32	35
3	47	Saint Bernard	24.6	45	105
4	49	Dobermann pinscher	25.8	45	35
5	40	Great Dane	26.5	55	90
6	37.5	Mixed breed	9.8	n/a	n/a

Abbreviations: n/a, not available; TAMIS, transanal minimally invasive surgery.

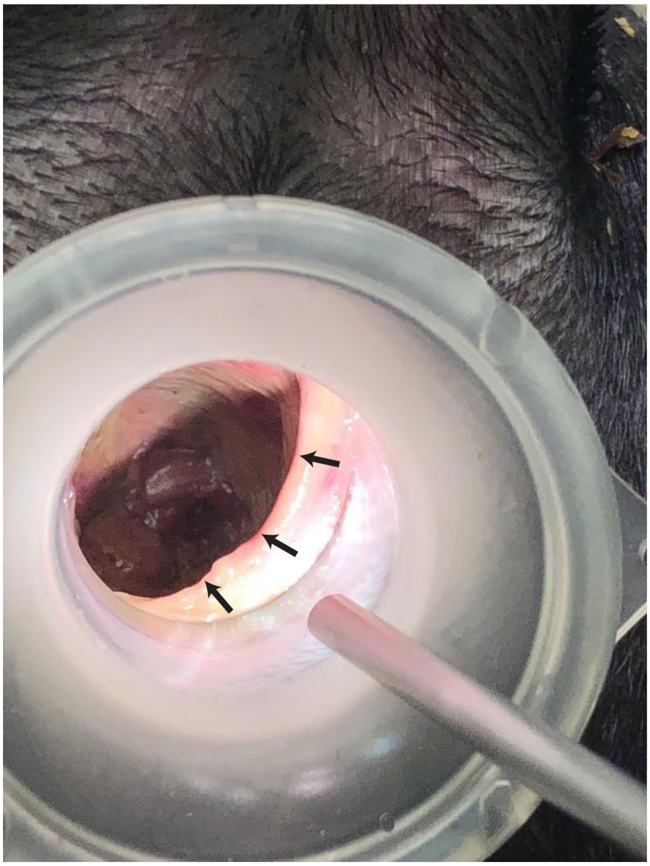

FIGURE 4 In one dog, a persistent fold of rectal mucosa (arrows) protruded over part of the inner part of the sheath and sustained minor iatrogenic trauma during instrument exchanges during the procedure in this case

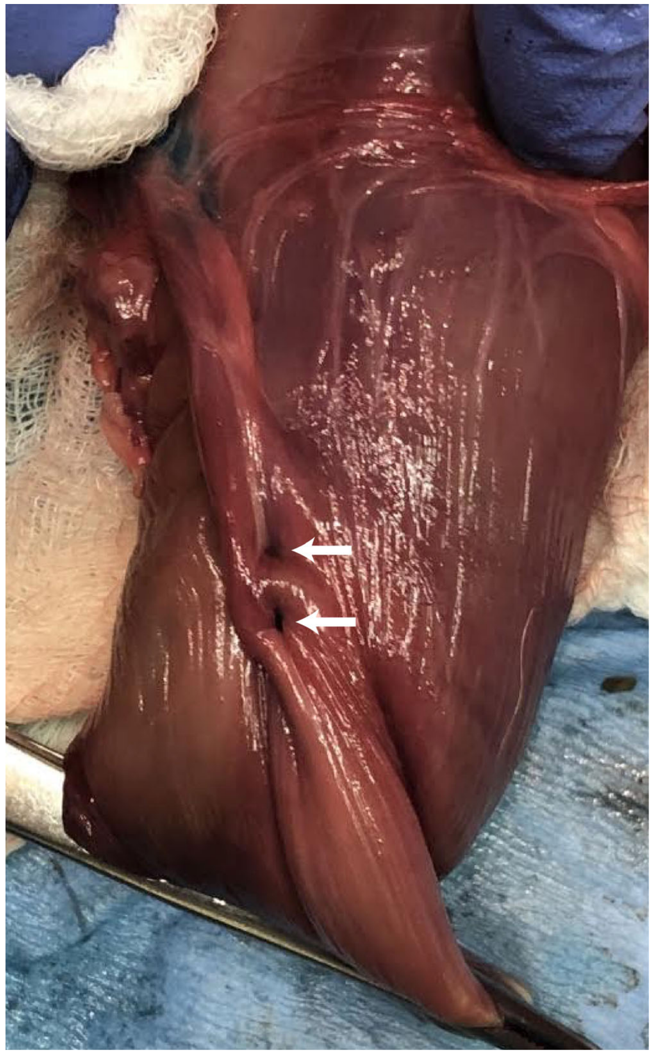

FIGURE 5 In this specimen, two suture bites (arrows) that penetrated through to the serosal layer during suturing of the defect can be seen on the serosal side

3.4 | Assessment of peritoneal penetration, leakage, and resection location

There was no gross evidence of full-thickness penetration of the rectum. In one dog, very mild leakage of the methylene blue dye could be seen emanating from one of the needle holes, and, in five dogs, some methylene blue could be seen dissecting between the planes of the rectal wall without evidence of leakage. Inspection of the suture

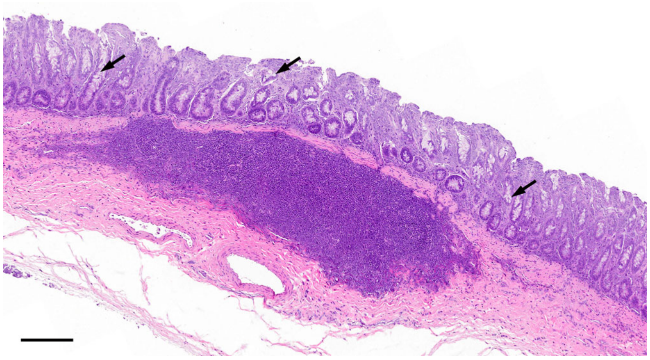

FIGURE 6 Rectal mucosa and submucosa. This section has an intact mucosal surface subtended by a large lymphoid aggregate and abundant submucosa. Epithelial cells are missing from the luminal surface due to autolysis, but abundant goblet cells (arrows) are still evident within the glands. Hematoxylin and eosin stain. Scale bar = 0.25 mm

line from the mucosal side revealed no obvious defects in the mucosal closure. The median length of the suture line in the five of six dogs in which it was measured was 45 mm. The median distance from the aborad extent of the suture closure line to the anocutaneous junction in the five of six dogs in which it was measured was 35 mm (range, 35-105).

3.5 | Histology of resected rectum

All sections included good representation of mucosa, muscularis mucosa, and submucosa, with lymphoid tissue present in three samples (Figure 6). Histology sections for all but one sample were on a plane perpendicular to the surface. The length of samples varied from 9.8 to 26.5 mm, with a median length of 24.5 mm. The sections extended a significant depth through the submucosa.

4 | DISCUSSION

The results of this study provide evidence that TAMIS could be a feasible replacement for rectal pull-through in a subset of dogs with rectal tumors. The rectal pull-through procedure has been found to be a morbid procedure in dogs with high rates of postoperative fecal incontinence and other serious complications.[6,7] Therefore, TAMIS may represent a significant therapeutic advancement for some dogs if the technique demonstrated in cadavers in this study can be performed safely in dogs with naturally occurring disease.

Case selection is of critical importance when patients are considered for any minimally invasive intervention. In human, rectal adenoma (polyp) resection is usually performed in the submucosal plane and has been associated with a low (<5%) incidence of incomplete resection and local recurrence.[14-16,18,19] Full thickness resection has been more recently reported for more proximal and malignant lesions, including full-thickness resections taken with a 1-cm full-thickness margin by using TAMIS.[16,18,19] If TAMIS is to be introduced for resection of canine rectal masses, it will be essential to understand its advantages and limitations as well as the anatomic differences between human and dogs that must be taken into account when the procedure is considered. First, the rectal wall is very thin in dogs, measuring approximately 3 mm in the midpelvic area level and as thin as 1 mm when distended.[20] The corresponding measurements of normal colonic thickness in human are 2 to 3 mm.[21] Second, human and dogs have significantly different rectal anatomy with regard to the peritoneal reflection. In human, the rectum is approximately 15 to 20 cm long, with only the cranial third being covered in peritoneum, leaving a length of approximately 8 to 12 cm of retroperitoneal rectum.[22] In dogs, the peritoneal reflection is much more caudally located within the pelvis, leaving only 2 to 3 cm of retroperitoneal rectum.[23] Therefore, any full-thickness perforation or resection will result in peritoneal penetration in dogs at almost any level of the rectum apart from the terminal 2 to 3 cm, whereas, in human, penetration of the caudal 8 to 12 cm of rectum will result only in entry to the retroperitoneal space. Because peritoneal penetration can lead to septic peritonitis, this complication could be associated with greater morbidity compared with retroperitoneal penetration alone.

Because of the limitations of the TAMIS technique and the anatomical differences between human and dogs, it will be critical to evaluate case selection criteria for TAMIS in dogs carefully. The authors of this study suggest that, initially, rectal polyps restricted to the mucosa of the rectal wall in the mid- to orad third of the rectum will be the best candidates for the TAMIS procedure. If the submucosal dissection plane used in this cohort of cadavers is used for resection of lesions that have malignantly transformed and invaded into the deeper aspects of the submucosa or muscular layers of the rectum, TAMIS will most likely result in incomplete resection and local recurrence. Deeper dissection may also increase the risk of full-thickness penetration, which could lead to peritonitis and/or tumor dissemination into the peritoneal cavity because of the mostly peritoneal location of the canine rectum. It may be that, as experience with TAMIS increases, more advanced and more extensive lesions can be successfully resected. However, it is generally wise in the early part of the learning curve with any novel minimally invasive approach to restrict use to the cohort of modestly sized lesions that are less extensive and less invasive.

The dimensions of the GP-TAP device also represent a significant challenge for the performance of TAMIS in any dogs other than large and giant breed dogs because the device is designed for the human rectum and is large. In the smallest dog in this study (37.5 kg), placement of the device was associated with significant tension, and the authors were unable to easily prevent a fold of rectal wall protruding from the end of the inner sheath and causing some interference with visualization (Figure 4). It is, however, worth noting that epithelial tumors of the rectum occur more commonly in larger breeds of dog, with a median weight of 26.2 kg quoted in one large cohort of dogs reported in the literature.[24] The ease-of-placement scores in this study were all low (1 or 2 of 5), providing evidence that, in large dogs such as those used in this study, the GP-TAP was easily placed. Smaller GP-TAP devices are not currently available. The first descriptions[13] of TAMIS used the SILS port (Medtronic), which is a significantly smaller device than the GP-TAP used in this study that may allow the procedure to be performed in slightly smaller breeds. However, in small dogs, even if smaller devices were available, it is difficult to predict whether sufficient working space would be available to perform the resection and suture the defect by using the techniques described.

The TAMIS technique reported in this study was technically challenging and is likely to be more challenging in a clinical animal that has intact peristalsis and will bleed when incised. However, the authors consider the technique translatable into the clinic for surgeons who have some experience in intracorporeal suturing and the availability of the GP-TAP. After practice in one canine cadaver prior to study initiation, the authors ascertained that, to be in the plane deep to the mucosa and submucosa but superficial to the muscular layers, the dissection plane had to be surprisingly superficial (Figure 2). Although it was not used in this study and is not a standard part of TAMIS procedures in human, injection of saline submucosally may have aided in raising the more superficial layers of the rectal wall and facilitated identification of the submucosal plane, as was performed in a previously described case report of endoscopic resection and snare polypectomy in a dog.[10] This technique has recently been described in human as a modification of TAMIS, but extensive evaluation of its advantages and disadvantages is currently lacking in the human literature.[25] It is very easy to penetrate through the deeper muscular layers and into the peritoneal cavity if an aggressive dissection is performed. After the initial incision through the mucosa was made, it was relatively easy to find the correct dissection plane under the mucosa/submucosa within which to dissect. All dogs in this study were surgically treated in sternal recumbency, and, in all cases, a sample from the ventral rectal wall (6 o'clock position) was resected. The authors concluded that operating with a 30° telescope and instruments going through the lower ports of the GP-TAP made resection of rectum at the 6 o'clock position technically easier. In cases in which the lesion is on the lateral or dorsal wall, a human patient would be positioned appropriately to allow mass positioning at the 6 o'clock position without regard to the positioning of the mass within the rectal circumference. As an example, a human with a polyp located on the left lateral rectal wall would likely be positioned for TAMIS in left lateral recumbency, allowing the lesion to be maintained at the 6 o'clock position in relation to the surgeon's view. In a canine clinical case, the authors would recommend taking the same approach to positioning to maintain correct orientation.

Closure of the defect was performed by using a simple continuous closure pattern in an orad to aborad orientation and was greatly facilitated by the use of barbed suture material. Barbed suture obviated the requirement for knot tying at either end of the suture line and probably contributed greatly to the ability to close the defect in a relatively short time (median defect closure time was only 17 minutes). The use of a very small needle, such as the 17-mm CV-23 needle used in this study, also facilitated suture bites being taken in the very limited space provided within the rectal lumen of these canine cadavers. Although dog weight varied from 37.5 to 60 kg, we found suturing to be very feasible within the working space afforded by a pneumorectum of 8 to 10 mm Hg. The requirement for closure of the rectal wall defect has been questioned in the human literature by some authors; leaving the defect open has been found not to be associated with an increase in complications.[26] The authors of the study reported here elected a cautious approach because of the possibility of creating a full-thickness defect in the thin wall of the canine rectum and elected to close the defect. Until the technique has been more critically evaluated in additional studies, defect closure will likely remain our approach of choice when we use TAMIS in clinical animals.

Lesion location is a very important determinant of technique choice in canine rectal resection. The mid- to cranial rectum is encased in the boney pelvis and is not easily accessible. The degree to which these areas of the rectum can be accessed by mucosal eversion and pull-through techniques is highly variable and difficult to predict preoperatively in our experience. One potential advantage of TAMIS is that access to the mid- and cranial rectum may be feasible because of the long reach of the instrumentation. In this study, the median distance from the aborad extent of the suture closure line to the anocutaneous junction was 35 mm but was as much as 105 mm in one dog, providing evidence that access to the cranial rectum or even terminal portions of the descending colon may be possible in some dogs. In addition to the ability to reach rectal tumors in more cranial locations, one of the major challenges is achieving a clean margin of resection in these cases. A recent report of a retrospective study on rectal pull-through in dogs described the incidence of dirty margins 35%.[7] Obtaining a negative margin orad to a lesion with a rectal pull-through can be challenging, and techniques that afford greater access to the lesion may improve the ability to obtain a negative margin. Studies in live dogs with naturally occurring disease are required to document the incidence of clean resection to evaluate this hypothesis.

This study has several very important limitations. A cadaveric project cannot emulate the challenges that will be faced when this technique is transferred to the clinic in dogs with rectal masses. A small cohort of only six cadavers was used, and different complications may have occurred had a larger cohort of animals been used. We performed manual removal of fecal material from the rectum only, which may not be as effective in live dogs who have functional peristalsis occurring intraoperatively and will likely have a less complete rectal cleansing and, therefore, a more obscured visual field. Other limitations of this procedure include the high cost of the GP-TAP device, which is single-use and disposable. Because of the gel-like material in the Gel-seal cap, it is unlikely that this device can safely be resterilized to use it in more than one animal.

In conclusion, this study provides promising results for rectal submucosal resection in a small cohort of large breed canine cadavers. If this technique can be successfully translated into the clinic for treatment of client-owned animals with epithelial lesions of the rectal wall that have not penetrated into the deeper layers, it may represent a much less morbid minimally invasive technique compared to rectal pull-through for resection of these masses.

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AUTHOR CONTRIBUTIONS

Mayhew PD, BVM&S: co-conceived study, performed surgical procedures, manuscript preparation; Balsa IM, DVM: co-conceived study, performed surgical procedures, manuscript preparation; Guerzon CN: assisted with surgical procedures, manuscript preparation; Gibson EA, DVM: assisted with surgical procedures, manuscript preparation; Keel MK, DVM, PhD: performed histologic analyses, manuscript preparation; Brun MV, DVM, MSc, PhD: co-conceived study, manuscript preparation; Lillo Araya FJ, DVM, DIM: co-conceived study, manuscript preparation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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