—Original Article—

Endoscopic ultrasound-guided inoculation of transmissible venereal tumor in the colon: A large animal model for colon neoplasia

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ABSTRACT

Background: To develop and evaluate the feasibility of emerging interventions, animal models with accurate anatomical environment are required. **Objectives:** We aimed to establish a clinically relevant colorectal tumor model with canine transmissible venereal tumor (CTVT) utilizing endoscopic ultrasound (EUS) imaging guidance. **Design:** Survival study using a canine model. **Setting:** Endoscopic animal research laboratory at a tertiary cancer center. **Materials and Methods:** This study involved five canines. **Interventions:** A colorectal tumor model was established and evaluated in five canines under cyclosporine immune suppression. Under endoscopic imaging guidance, saline was injected into the submucosal layer forming a bleb. Subsequently, CTVT was inoculated into the bleb under EUS guidance. Endoscopy was the primary method of assessing tumor growth. Tumors developed in 60-130 days. Upon detection of lesions >1 cm, the animals were euthanized and the tumors were harvested for histopathological characterization. **Main outcome measurements:** Success rate of tumor growth. The presence or absence of vasculature inside tumors. **Results:** Colorectal tumor successfully developed in three out of the five animals (60%). Among the ones with tumor growth, average inoculated CTVT volume, incubation time, and tumor size was 1.8 cc, 65.7 days, and 2.0 cm, respectively. The two animals without tumor growth were observed for >100 days. In all the tumors, vascular structure was characterized with CD31 imunohistochemical stain. **Limitations:** Small number of animals. **Conclusion:** We succeeded in creating a new colorectal tumor canine model with CTVT utilizing EUS.

Key words: Animal tumor model, canine transmissible venereal tumor (CTVT), colorectal cancer, endoscopic submucosal dissection (ESD), endoscopic ultrasound (EUS), natural orifice transluminal endoscopic surgery (NOTES), tumor model

INTRODUCTION

Colorectal cancers (CRCs) are the third most common malignant neoplasm worldwide and the second leading

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cause of cancer deaths in the United States.[1,2] Over the last decade, the overall incidence and mortality of CRCs has been slowly declining. This has largely been attributed to the increase in effective screening for early detection as well as the increasing efficacy of interventional treatment (e.g., surgery, endoscopic treatment) and systemic chemotherapy.

Endoscopic removal of polyps (i.e., polypectomy) is primary and a quite effective interventional treatment for removing colorectal adenoma or cancer localized in the mucosal layer to minimize the risk of developing advanced cancer.[3,4] Minimally invasive endoscopic alternatives [e.g., natural orifice transluminal endoscopic surgery (NOTES), endoscopic submucosal dissection (ESD), radiofrequency ablation, and laser ablation] for specific clinical indications have been developed as alternatives to conventional open or laparoscopic surgery.[5-9] In addition, several emerging therapuetic techniques [e.g., nanotechnology-mediated therapies,[10-14] high intensity focused ultrasound (HIFU)[15,16]] have the potential to be applied for the local management of CRCs.

Often, these emergent procedures are rigorously investigated and refined in animal models prior to initial clinical trials. Regarding preclinical endoscopic treatment investigations, artificial lesion can be created by simple submucosal injections forming blebs with normal saline and/or hyaluronic acid.[8,17] Moreover, it requires laparotomy to implant artificial substances[18,19] or anatomical reconstruction mimicking tumors.[20] However, such models lack the essential anatomical similarity (i.e., blood supply and connection with surrounding tissues) for an accurate preclinical safety and feasibility study. Animal models that can better simulate the anatomical environment of interest and provide tissue for histopathological analysis are desired.

In this respect, the canine colon provides an excellent approximation to the human colon in both shape and size and is therefore, deemed to be favorable for the accommodation of human sized interventional equipment. Additionally, the canine transmissible venereal tumor (CTVT) model has been used to investigate interventions in various organs, including the skin, lung, prostate, bone, and brain, with great success.[21-26] We aimed in this study to investigate the feasibility of establishing a colorectal tumor model utilizing CTVT simulating a more precise anatomical environment for feasibility studies of preclinical interventional treatments.

MATERIALS AND METHODS

All of the research was conducted in accordance with the approval of our institution's Animal Care and Use Committee guidelines. Male mongrel dogs (n = 5) weighing 20-30 kg were placed on an immunosuppressive regimen [cyclosporine; 10 mg/kg *bis in die* or twice a day (b.i.d.)] 2 weeks prior to inoculation and moved to a regimen of 10 mg/kg once daily (o.d.) after inoculation. Bowel preparation began 2 days prior to inoculation. The feed was restricted to one can of food, mixed with five tablets of sodium phosphate (Visicol) to help empty the rectum. Twenty-four hours prior to inoculation, 15 tablets were combined with the canned food and two water enemas were administered on the day of the inoculation procedure. In the first two animals, a polyethylene glycol (PEG) 3350 solution (NuLYTELY; 30 mL/kg) with electrolytes solution was employed as a bowel-cleansing agent. The resulting distress and adverse effects associated with this procedure required premature euthanasia of two animals.

For tumor inoculation, the animals received a preanesthetic [10 mg/kg acepromazine intramuscularly (IM) and 1.2 mg/kg atropine IM] followed by 15 mL/kg pentothal IV for induction and 1% isoflurane for maintenance. CTVT, which had been freshly harvested from severely compromised immunodeficient (SCID) mouse xenographs and frozen for storage, was inoculated into the colonic mucosa approximately 10-30 cm rostral to the rectum under endoscopic ultrasound (EUS) guidance (Olympus GF-UC30P, Olympus America Inc., Melville, New York, USA) as follows. The structure of the colon wall (mucosa, submucosa, *muscularis propria*, and *serosa/adventitia*) was visible on the EUS images. Given the 3-4 mm total thickness of the mobile colorectal wall with a thin ~1 mm echogenic submucosal layer, it was not possible to precisely position an EUS-guided fine-needle aspiration (FNA) needle tip into the submucosa. We therefore, used a standard endoscope (GIF-H180, Olympus America Inc., Melville, New York, USA), and a 2.3-mm disposable injector with a 5-mm long 25G needle (NM-200U-0525; Olympus America Inc., Melville, New York, USA) was used to puncture the mucosal layer at the inoculation site, thus creating a submucosal bleb similar to the standard techniques used during saline-assisted polypectomy and endoscopic mucosal resection. The bleb was created by injection of approximately 20-25 mL of sterile saline [Figure 1a]. The CTVT injectate was too thick and viscous to

be injected into the submucosa with a standard 25G endoscopic injection needle. Thus, the standard endoscope was removed and the linear echoendoscope (Olympus GF-UC30P) was inserted into the colon/rectum. The blebs were visible on the ultrasound imaging as round/oval anechoic masses within the submucosa. A 19G EUS-guided FNA needle (Echotip Ultra; Cook, Bloomington, Maryland, USA) was inserted into the bleb under EUS guidance to control needle penetration into the bleb [Figure 1b], with the tip of the 19 G needle seen in the center of the submucosal saline bleb. Freshly prepared CTVT (1.5-2.0 cc) was injected into the bleb and the injectate was visible on ultrasound as an echogenic mass within the anechoic bleb [Figure 1c]. The injectate was visible endoscopically as a whitish discolored area through the transparent mucosal wall (Figure 1d, GIF-H180, Olympus America Inc., Melville, New York, USA). After inoculation, the *mucosa* adjacent to the injection site was tattooed with SPOT ink (GI Supply, Camp Hill, Pennsylvania, USA) with an injection needle for future identification during follow-up endoscopy to assess tumor growth. For dogs 1, 3, and 4, subcutaneous tumors (0.5-1.0 cc) were also implanted in the paraspinal muscle region with the goal of providing fresh tumor fragments for the next round of inoculations as well to confirm the viability and to evaluate the potential of an external surrogate marker indicative of tumor growth.

Lesions were allowed to develop for approximately 60-100 days with the animals remaining on cyclosporine for the duration. Endoscopic visualization of the tattooed sites was the primary means of assessing colonic transmissible venereal tumor (TVT) development *in vivo*. Upon detection of lesions ≥1 cm, the animals were euthanized and the tumors harvested for histopathological characterization, which included hematoxylin and eosin (H&E) staining and selected applications of CD31 staining to evaluate tumor vasculature.

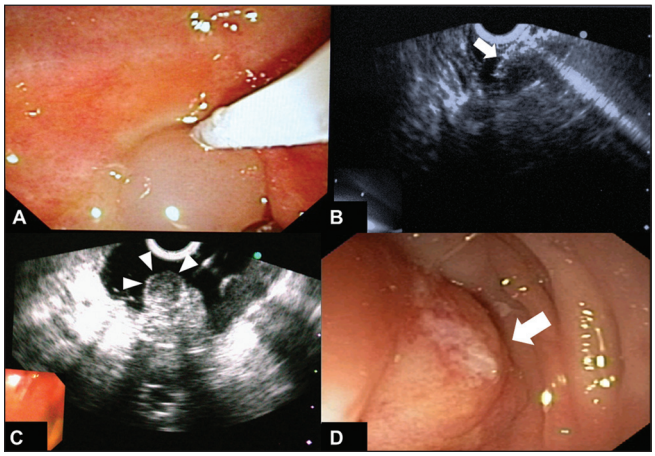

Figure 1. Tumor inoculation. (a) Saline bleb creation: A 25G injection needle was used to puncture the mucosal layer at the inoculation site (b) Inoculation needle inside bleb: The needle as well as the bleb were visible on the ultrasound image (arrow) (c) Injection of TVT: Freshly prepared CTVT (1.5 ml-2.0 ml) was injected into the bleb. The injectate was visible on ultrasound as an echogenic mass within the anechoic bleb (d) Final tumor bleb: The injectate was also usually visible endoscopically as a white area through the transparent mucosal wall

RESULTS

A summary of the sites of inoculation, days of growth, and resulting maximum lesion diameters is found in Table 1. In the first animal (dog 1), TVT tumor was inoculated (1.0 cc) 10 cm rostral to the rectum. This site received a lower volume as it was a secondary injection. The primary bleb with 2.0 cc burst during injection and a subcutaneous tumor (1.0 cc) had already been inoculated. In this animal, no tumor growth was

Table 1. Summary of the inoculation sites, time, and tumor size

Dog	Inoculation site	Time	Size	Notes
1	Colon	130 days		
	10 cm rostral (2.0 cc)		–	No tumor growth
	10 cm rostral (1.0 cc)		–	
	Subcutaneous (1.0 cc)		–	
2	Colon			
	10 cm rostral (2.0 cc)		1 cm	+5 serosal metastases (<1 cm)
	Subcutaneous (none)		–	Extensive metastases
	Lung			
3	Colon	100 days		
	20 cm rostral (2.0 cc)		–	No tumor growth
	Subcutaneous (0.5 cc)		2.5 cm	Ulcerated tumor mass
4	Colon	79 days		
	12 cm rostral (1.5 cc)		2.0 cm	(No metastases)
	30 cm rostral (2.0 cc)		–	Visible on endoscopy
	Subcutaneous (0.5 cc)		–	Mucosal thickening
5	Colon	62 days		
	10 cm rostral (2.0 cc)		3.0 cm	Visible on endoscopy
	20 cm rostral (2.0 cc)		2.0 cm	Visible on endoscopy
	30 cm rostral (2.0 cc)		–	*Serosa* only
	40 cm rostral (2.0 cc)		–	Metastasis
	Colonic lymph node		–	
	Subcutaneous (none)			

observed 130 days after inoculation. The reasons for this result were unknown but could have potentially been unviable inoculate, not enough viable inoculate, or problems with reliable cyclosporine delivery. An attempt at a second inoculation was scheduled. However, respiratory adverse effects resulting from the polyethylene glycol (PEG)-based bowel preparation procedure resulted in the euthanasia of the animal. Gross and microscopic findings showed no evidence of TVT at the inoculation sites or in potential metastatic locations such as the lung or liver.

The second subject (dog 2) was inoculated (2.0 cc) at one colonic site only 10 cm rostral to the rectum. At 60 days, the animal was being prepared to evaluate the growth of the tumor inoculate. The PEG-based bowel preparation procedure was repeated, with great care being taken in the placement of the gastric tube to avoid any regurgitation of the PEG. However, this was not enough to alleviate adverse effects and the animal was euthanized prior to the procedure due to distress from the bowel preparation procedure.

At necropsy, the primary site of TVT inoculation resulted in a 1-cm diameter mass within the colonic wall [Figure 2]. On cut section, the tumor extended through the colonic wall with protrusion on both the mucosal and serosal surfaces. On H&E-stained tissue, the tumor was composed of solid sheets of round cells

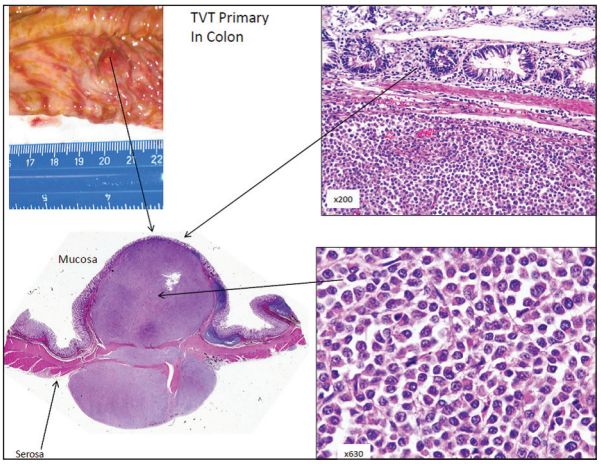

Figure 2. Pathology of dog 2: The single primary inoculate extended through the full thickness of the colonic wall, protruding into both the colonic lumen on the mucosal surface and the abdominal cavity on the serosal surface. The TVT expanded through the submucosa and muscular layers of the colon to grow as an invasive tumor. Tumor cells were visualized as solid sheets of round cells with distinct cell borders, abundant eosinophilic cytoplasm, and oval to round nuclei supported by a thin fibrovascular stroma. Mitotic figures were frequent. The liver was within normal limits but the spleen had a moderate loss of lymphoid cells from both the T- and B-lymphoid areas of the white pulp

with distinct cytoplasmic borders and elliptical nuclei of varying eccentricities. Tumor cells were distributed in a thin fibrovascular stroma with frequent mitotic figures. The TVT extended through the submucosa and muscular layers of the colon following a path of least resistance. In addition to the primary inoculate site, multiple (five) small (≤1 cm) independent TVT lesions were observed on the colonic serosal surface. None of these sites were lymph nodes and all were tumors with increased vascularity. In addition, the lungs were severely congested with diffuse miliary metastatic TVT accompanied by mixed inflammatory cells. The density of tumor/inflammation was mild to severe (grades 2-3 in the left lung and 3-4 in the right lung). No significant lesions or changes were observed in the liver but there was a moderate depletion of lymphoid cells from both the T- and B-lymphoid areas in the white pulp of the spleen. In this limited pilot study, this was the only animal that had extensive metastases to the lung and colonic *serosa*. CD31 stains showed fairly uniform vasculature throughout the primary transmural TVT tumor. The tumor did not demonstrate pockets of necrosis as might have been observed in larger CTVT tumors. Blood vessels in the adjacent submucosa and on the surface of the *serosa* were relatively large and dilated. Blood vessels within the tumor, for the most part, were small and oriented in a random pattern. In comparison, blood vessels in the adjacent normal *mucosa* were narrow, oriented vertical with respect to the luminal surface and located between colonic crypts. Blood vessels in the submucosa were characterized by a horizontal orientation with respect to the luminal surface. Blood vessels in the *serosa* were usually indistinct. While angiogenesis is a necessary component of tumorogenesis, the morphologic characteristics of most of the blood vessels within the TVT were those of "mature" vessels. However, some vessels in the mass did demonstrate tell-tale signs of angiogenesis [Figure 3].

The third subject (dog 3) was inoculated in the colon 20 cm rostral to the anus (2.0 cc) as well as subcutaneously (0.5 cc). Endoscopy of the inoculation site at 79 days did not reveal any developing tumor. After approximately 100 days after inoculation, no TVT was observed in the colon or other internal organs. However, a large, raised TVT lesion (2.5 cm) was present in the dorsal lumbar subcutaneous inoculation site. The animal was euthanized and the tumor harvested. The epidermis over the mass was extensively ulcerated; the bulk of the mass was located

in the epidermis and dermis with extension into the adipose tissue of the subcutis. The mass was solid and pale white on the cut section. On H&E, the neoplastic tissue was partially encapsulated and composed of tightly packed round cells with a moderate amount of eosinophilic cytoplasm. Groups of cells were separated by a fine fibrovascular connective tissue stroma. The neoplastic cells revealed cellular atypia and high mitotic activity (up to 15 per high power field). The periphery of the lesion was characterized by a zone of intense inflammatory cells in which lymphocytes predominated. As anticipated, the lesion was well-vascularized on CD 31 immunostains.

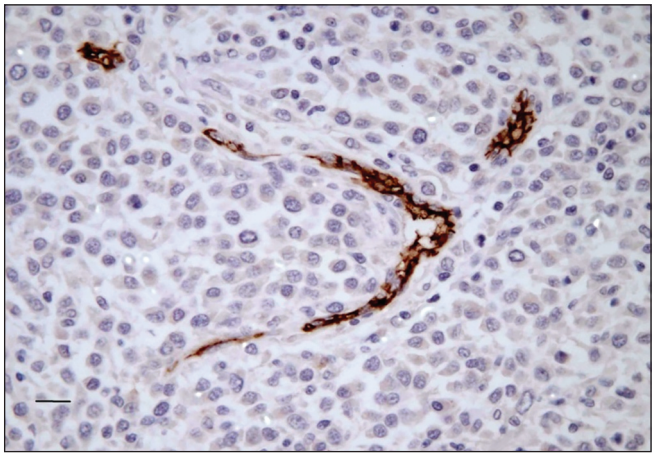

Figure 3. Dog 2 immunostaining (CD31 staining of TVT) - A ">"-shaped blood vessel in center of photo may be example of angiogenesis (magnification x630, bar = 10 μm). Endothelial cells are plump, and the lower portion of the ">" is tapered. With angiogenesis, a single layer of endothelial cells extends from the blood vessel, and a lumen develops as the vessel matures. Mature blood vessels are lined by flattened endothelial cells

The fourth subject (dog 4) was inoculated at two sites in the colon (12 cm rostral to the anus with 1.5 cc and 30 cm rostral to the anus with 2 cc) and subcutaneously (0.5 cc). In this case, after 79 days of development, a 2 cm mass was visualized using both computed tomography (CT) imaging and endoscopy at the 12 cm site [Figure 4a]. The inoculation sites at 30 cm and the subcutis did not develop into masses. These observations were confirmed at necropsy as was the absence of any distant metastases in the internal organs. Grossly, there was a broad based, polyp-like mass in the colon approximately 12 cm rostral to the anus measuring approximately 2 cm in diameter [Figure 4b]. The sectioned mass was tan to white with a solid consistency. In the region of the second inoculation, approximately 25 cm-30 cm rostral to the anus and extending for some 15 cm up the colon, there was moderate transmural thickening of the colonic wall with a diffuse fibrinous pseudomembrane covering the mucosal surface.

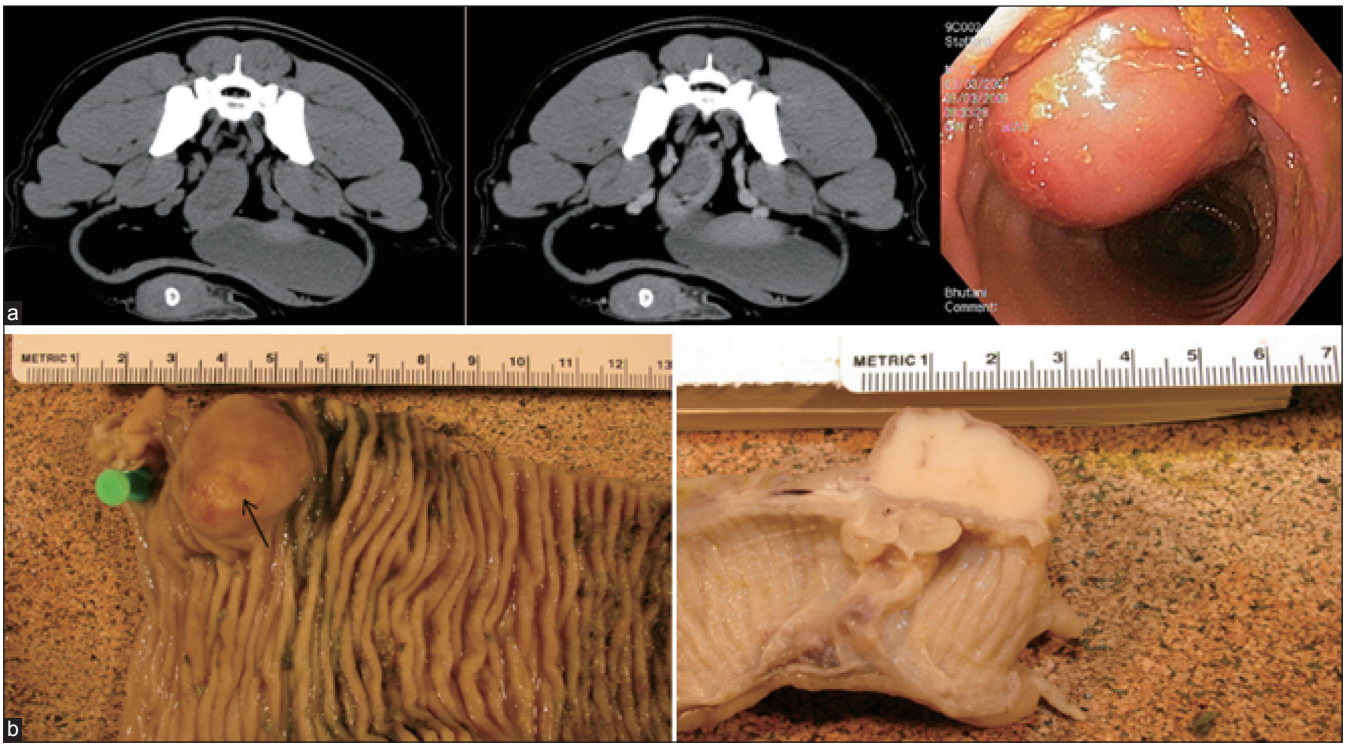

Figure 4. Dog 4 verifying lesion growth: (a) The lesion at 12 cm rostral to the rectum is visible on precontrast (left) and postcontrast (middle) CT images of the colon with Hounsfield units of 41 HU and 57 HU for the unenhanced and enhanced lesions, respectively. Endoscopy verified the results just prior to necropsy (right) (b) The tissues were formalin-fixed for 1 week. The mucosal surface of the tumor is ulcerated (arrow)

Histologic sections of the tumor mass, the proximal colon, and mesenteric lymph node were examined by the pathologist. Significant lesions were confined to the tumor and colon at the 12 cm inoculation site. The tumor mass was transmural and contained within the colonic *mucosa*, submucosa, and *tunica muscularis* with extension into the *serosa* at one site. Similar to the previous TVT samples, the mass was composed of tightly packed round cells with a moderate amount of eosinophilic cytoplasm, and groups of cells were separated by fine fibrovascular stroma. Again, the neoplastic cells revealed cellular atypia and high mitotic activity (up to 15 per high power field); CD 31 stains demonstrated that the lesion was well-vascularized. Sections of the abnormal colon had a moderate diffuse layer of fibrin and necrotic cellular debris covering the mucosal surface. The underlying *mucosa* was normal. Sections contained small submucosal aggregates of lymphoid cells with high mitotic activity. Morphologically, the cells within these nodules were more basophilic and on CD31 immunohistochemical staining, fewer blood vessels were observed. Therefore, these cells were differentiable from those seen within the neoplastic TVT mass.

The fifth subject (dog 5) was inoculated with 2 cc of TVT fragments at four sites within the colon (10 cm, 20 cm, 30 cm, and 40 cm rostral to the anus). There was no subcutaneous inoculation in this case. Endoscopy at 62 days after inoculation identified TVT in the colon at the 10-cm (3-cm) and 20-cm (2-cm) inoculation sites but none at the 30-cm or 40-cm inoculation sites [Figure 5]. The animal was euthanized and the tissues were harvested for pathological analysis. Gross pathology confirmed the presence of TVT masses in the *mucosa* and submucosa at the 10-cm and 20-cm locations [Figure 5]. No tumor mass was observed at the 30-cm location; however, the tumor was observed in the *tunica muscularis* and *serosa* at the 40-cm site likely due to an inoculation that had gone too deep. Microscopically, the mass at the 10-cm site was contained within the submucosa [Figure 6]. Lymphocytic infiltrates were present along the submucosal margin of the TVT. In addition, there was a focus of TVT on the serosal surface that was separate from the submucosal mass and a nearby lymph node that contained metastatic TVT cells [Figure 6]. The TVT mass at 20 cm was located within the submucosa but extended through breaks in the *muscularis mucosa* into the *mucosa* [Figure 7]. This may have resulted from the TVT inoculation from the

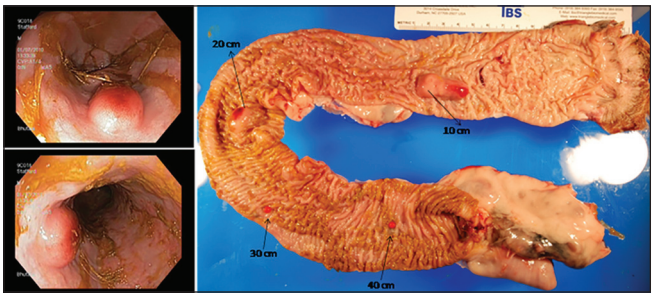

Figure 5. Dog 5 TVT tumors: Endoscopy on the day of necropsy revealed mucosal TVTs of 10 cm (~3 cm) and 20 cm (~2 cm) rostral to the anus; these were subsequently verified by histopathologic evaluation. A tumor confined only to the serosa was seen at the 40-cm marker, and no tumor was observed at the 30-cm inoculation site (red markers)

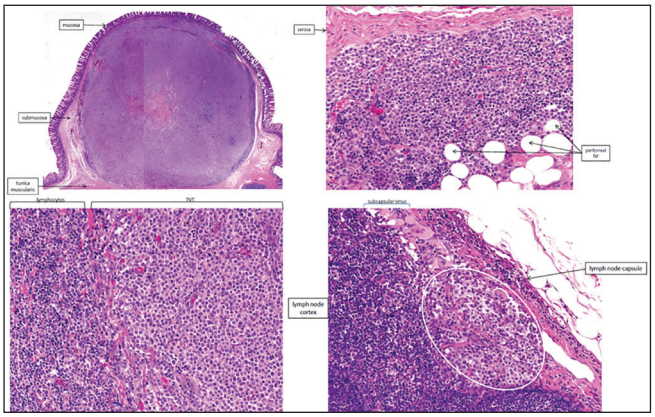

Figure 6. Dog 5 Pathology for TVT at 10 cm: The tumor is completely contained within the submucosa of the colon (magnification ×12.5) as shown in the top left pane. Below, a band of lymphocytes on the left is seen at the periphery of the tumor on the right (magnification ×200). In the top right, the TVT on the serosal surface is separate from the submucosal mass at 10 cm (magnification ×200) as shown in the top left pane. Below is a colonic lymph node with metastatic TVT in the subcapsular sinus (magnification ×200)

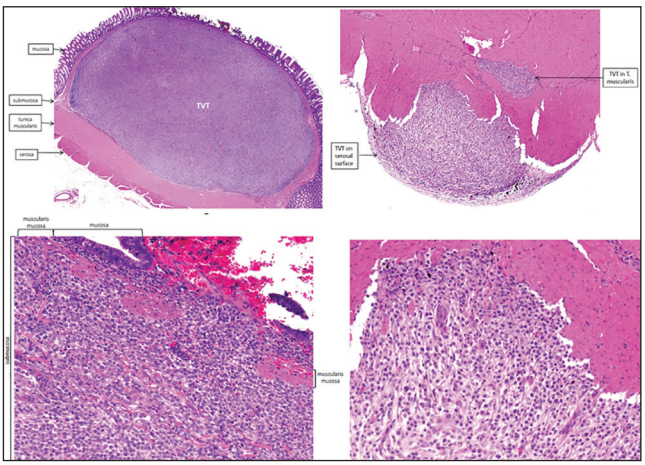

Figure 7. Dog 5 pathology: On the left, TVT at 20 cm (magnification ×12.5). Below, we see the TVT in the submucosa with extension through the *muscularis mucosa* and into the mucosa (magnification ×200). To the right, TVT at 40 cm is contained within the *muscularis propria* and *serosa* (magnification ×50); higher magnification of TVT cells on the serosal surface (magnification ×200)

colonic lumen. The TVT mass at 40 cm was completely contained within the *muscularis propria* and adjacent *serosa* [Figure 7].

DISCUSSION

The present study shows that EUS-guided submucosal inoculation of CTVT can successfully be used to develop a canine model of human colorectal tumors. This tumor model shows vascular structure as well as connection with surrounding tissues. Given an excellent approximation of canine colon to the human colon in both shape and size, we believe that this model will be suitable for accurate evaluation in the preclinical research of colorectal tumors.

There are several advantages in our tumor model. First, this study is the first study utilizing EUS to create a real tumor model in a luminal organ. This technique can be less invasive than previous large animal tumor models with laparotomy.[18-20] Furthermore, we believe that EUS-guided technique will provide accurate inoculation of tumor cells into the targeted locations and be applicable for other intraluminal or extraluminal tumor models (e.g., esophagus, stomach, and pancreas). Further refinements and reproducibility of our initial experience with this model development could provide a reliable, novel technique to deliver tumor tissue into the submucosal space. We initially attempted to inject CTVT into the submucosal layer utilizing a standard injection needle with endoscopic guidance (without EUS) but had difficulty making a sufficient and reliable bleb. It seemed that the injectant was too thick to inject through the 25G needle and the small amount of TVT injection (2-cc) was not enough to create an endoscopically visible bleb. Therefore, we modified the technique using EUS guidance. With this technique, we first created a submucosal saline bleb using endoscopy and a standard injection needle. Then using EUS, we could clearly see this bleb as an anechoic lesion in the submucosa, which was then targeted with a 19G EUS FNA needle that was planted in the center of this anechoic lesion resulting in precise injection of CTVT into the submucosal layer.

Second, as we had hoped, this model produced well-vascularized tumors, which were easily visible with endoscopy and CT. This anatomical approximation for human colorectal tumor could be a suitable model for research in endoscopic- and image-guided interventions. For example, Moss *et al.* studied *en bloc* resection rate of endoscopic mucosal resection (EMR) of normal porcine colonic *mucosa* by circumferential submucosal injection of succinylated gelation.[27] An actual tumor model such as the one developed by us can be potentially very useful for research and the teaching of minimally invasive techniques (such as NOTES, laparoscopic colon cancer resection, and endoscopic full thickness resection of gastrointestinal stromal tumors) by ensuring that a complete tumor resection is done with a clear margin devoid of tumor cells, simulating R0 tumor resection based on surgical principles. Moreover, this model has the potential to be utilized in research for other emerging imaging techniques. Multicolor fluorescent intravital live microscopy,[28] flexible multispectral scanning fiber endoscopy,[29] biochromoendoscopy with capsule endoscopy,[30] and multispectral endoscopic imaging[31] are emerging endoscopic imaging techniques, which may be potentially studied for gastrointestinal (GI) tumor imaging using our model. Voermans *et al.* compared transcolonic NOTES *versus* laparoscopic peritoneoscopy for the detection of peritoneal metastases by stapling small beads into the peritoneum to create artificial metastatic lesion.[32] Our finding of extensive metastases in one animal in the *serosa* and the lung could be potentially developed further to perform studies on the detection of distant metastases to compare the diagnostic accuracy of different and emerging minimally invasive imaging techniques. Preclinical studies for advances in cross-sectional tumor imaging (e.g., studies related to advances in CT, magnetic resonance image, and positron emission tomography) can also be evaluated considering its anatomical approximations to colorectal tumor. Many of these studies have been conducted *ex vivo* or *in vivo* with small animal models. Large animal tumor models such as ours could be used to narrow the gap between bench and bedside applications.

Johnson and Fleet recently published a good review of animal models of colorectal cancer.[33] They described three broad categories of animal models of colorectal neoplasia. These include chemically or environmentally induced cancers in rodents and cancers induced by genetic manipulation in mice. Rodents and mice are not suitable for endoscopic- or image-guided intervention type research due to their small size and adult endoscopic instruments that do not accommodate them. The third category is of spontaneous intestinal cancers in various large animal species that includes dogs who develop colorectal cancers that are similar to a great degree to that of humans but the limitation

is the low prevalence (<1%) of colorectal cancer in the pet dog population. Sheep and cats also develop spontaneous intestinal carcinoma but a majority of these are located in the small intestine and not in the colon. Cotton tap tamarin is a nonhuman primate that spontaneously develops ulcerative colitis and resultant adenocarcinoma. However, there is a long latency for the development of carcinogenesis.

The advantage of our large animal model compared to the above is that we do not need to wait for spontaneous development of tumors that can take years or may have a low prevalence (e.g., <1% in domesticated canines) and can induce colon cancer type tumors that can simulate similar anatomical structures to humans as well as evaluate devices usually used in humans before starting a clinical trial of new imaging or imaging-guided technical procedures. However, further technical refinement is required for this model to be used robustly in a study of image-guided interventions. This technique provided the most success with tumor inoculations ≤20 cm rostral to the anus (4/7, 57%). Of a limited three attempts, none of the deep seated tumor inoculations (≥30 cm) were successful with the exception of a serosal mass. This could simply be due to technical difficulty in inoculating in the deeper regions or it could be related to the diet or colonic physiology of the animals. For future studies, we suggest that tumor inoculations be ≤20 cm rostral of the anus to improve tumor acquisition. Additionally, the depth and size of the tumor varied in each site even in the same animal. Multimodality observation, including cross-sectional imaging, endoscopy, and EUS, will be feasible with this model for imaging and therapeutic technique development and reproducibility. For instance, localized small tumors within the submucosal layer may be reasonable models for ESD evaluation and deeper and bigger lesions for NOTES with full-thickness resection of the colorectal wall. Given the rate of growth observed in these animals, observations should begin within 4-6 weeks if the desire is to limit the size of the lesions to approximately 1 cm.

CONCLUSION

In conclusion, we have shown the feasibility of a colorectal tumor model utilizing CTVT in canines with EUS-guided injection of tumor cells. This model will be valuable in the continuing development of emerging less invasive locoregional treatment as well as imaging studies for colorectal tumors. Further studies are required to standardize, reproduce, and control more accurate tumor development in this model.

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Conflicts of interest

There are no conflicts of interest.

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