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Feasibility of combined upper and lower gastrointestinal endoscopic biopsy in the common marmoset (*Callithrix jacchus*) to evaluate gastrointestinal diseases

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Abstract

Background: Chronic gastroenteropathies, including gluten sensitivity and marmoset wasting syndrome, frequently occur in captive colonies of common marmosets (*Callithrix jacchus*). Early identification and diagnosis of affected animals are desirable. Endoscopic examination of the colon in marmosets is described, but the small intestine can harbor significant mucosal lesions not representing those in the colon. Evaluating the small intestine currently requires invasive surgical biopsies due to the small patient size, carrying a risk of severe complications.

Methods: Endoscopic intubation and multisite biopsy of the duodenum/proximal jejunum are demonstrated in 10 marmosets under general anesthesia.

Results: Esophagogastroduodenoscopy with colonoscopy efficiently aid in examining the gastrointestinal tract and obtaining an antemortem histologic diagnosis in marmosets with chronic gastrointestinal signs.

Conclusions: This minimally invasive technique is feasible in marmosets. Future investigations into the pathogenesis of chronic gastroenteropathies will benefit from these data, leading to improved animal welfare and better individual and colony health management.

KEYWORDS
anesthesia, biopsy forceps, duodenum, endoscopy, histopathology, intubation, marmoset monkey

1 | INTRODUCTION

Captive colonies of the common marmoset (*Callithrix jacchus*) have a high prevalence (28–60%) of chronic gastrointestinal conditions, including gluten sensitivity [1] and the complex of marmoset wasting syndrome (MWS) [2–4], which is characterized as a spectrum of disease phenotypes carrying high morbidity and mortality rates [5–7]. Early identification and diagnosis of affected animals are paramount for the optimal health management of individual marmosets and maintenance of captive marmoset colonies. Apart from the impact on animal health, both "simian gluten sensitivity" and MWS have been proposed as spontaneous animal models of human inflammatory bowel disease [6,8,9]. Furthermore, a recent study reported that MWS resembles chronic inflammatory enteropathies (with or without gastrointestinal protein loss) in dogs and cats [8], which also serve as spontaneous models of human inflammatory bowel disease [10].

Diagnosing and further characterizing gluten sensitivity, MWS, and other gastrointestinal conditions (e.g., neoplasms such as lymphoma) in marmosets requires a tissue diagnosis—usually obtained postmortem—in addition to non-invasive diagnostics including routine blood work, urinalysis, fecal examination, diagnostic imaging, and further specific serum tests (e.g., hormone levels and specific titers to dietary antigens) [1,2,6,8]. Previous studies on chronic gastrointestinal disease and MWS in marmosets, which also included endoscopic examination antemortem, have focused on evaluating the large intestine for the presence of inflammatory and/or morphological lesions [2,3,6,11,12]. However, recent reports suggest that the small intestine often harbors significant mucosal lesions, which are not necessarily representative of the disease process or lesion severity in the large intestine [4,7,8] and can also present varying disease phenotypes [7,13]. Hence, these lesions cannot be detected and biopsied for an antemortem tissue diagnosis if only the large intestine is evaluated. Particularly, the comparison with human celiac disease, presenting an inflammatory condition of the small intestine [14], warrants further evaluation of the small intestine in marmosets with chronic gastrointestinal signs [1,11] and suspected "simian gluten sensitivity" [1].

Antemortem diagnostics in marmosets with chronic gastrointestinal signs, particularly the presumptive diagnosis of MWS and further investigation or characterization of gluten sensitivity, remains challenging. This can result in a delay of more invasive diagnostics and affected animals being diagnosed at an advanced stage of the disease, which can present a significant problem in research colonies of marmosets. In addition to clinical and clinicopathologic tests, minimally invasive diagnostics to document and further investigate small intestinal mucosal lesions are desirable. Endoscopic evaluation of the esophagus, stomach, and colon has been reported in a few marmosets [15,16]. However, evaluating the small intestine in this species is currently still believed to require more invasive surgical biopsies either via laparotomy or laparoscopic techniques due to the small patient size [1,4,16]. These invasive diagnostics are associated with an increased risk of severe complications (e.g., suture dehiscence), particularly in hypoalbuminemic patients. For reasons including improved animal welfare and better individual and colony health management, less invasive diagnostics are highly desirable tools in clinical practice and research. Here, we demonstrate for the first time successful endoscopic intubation and multisite biopsy of the small intestine (duodenum and proximal jejunum) in a small group of marmosets under general anesthesia.

2 | MATERIALS AND METHODS

2.1 | Animals

A total of 10 marmosets with chronic gastrointestinal signs were included in this retrospective case series (6 females, 4 males), ranging from 4.5 to 15 years of age and weighing between 340 and 428 grams (12–15 oz). All animals had a gastrointestinal endoscopy performed for diagnostic purposes, and some of the animals were euthanized for medical reasons and further diagnostic investigation of the underlying pathology. One male animal underwent repeated endoscopy 14 months apart: the first endoscopy for further diagnostic evaluation of mild gastrointestinal signs and repeated endoscopy when showing a worsening clinical condition with severe, chronic gastrointestinal signs. All animals were housed in the same vivarium at the University of Leipzig College of Veterinary Medicine's primate research center (Institute of Physiological Chemistry) as previously described [1,17]. Briefly, in addition to ample opportunities for social interaction, the standard of care included regular physical, parasitological, and microbiological examinations. Water was available at all times, and all marmosets were fed twice daily: a mashed meal in the morning, gluten-free marmoset pellets in the afternoon, and a selection of fruits and/or vegetables [1]. Ethics approval for the further investigation of chronic gastrointestinal diseases in marmosets, which complies with the German animal welfare regulations, was obtained after independent review (#TVV 55/16) from the local authority for animal welfare (Regional Council of the State of Saxony, Leipzig/Chemnitz, SN, Germany).

2.2 | Preparation for endoscopy

The morning prior to upper and lower gastrointestinal endoscopy, marmosets were given a warm-water enema for rectocolonic cleansing. Food was withheld for 16 hours prior to the procedure, whereas access to water was available until approximately one hour before endoscopy.

2.3 | General anesthesia

All animals were pre-medicated with a combination of Goettinger Mixture II (GM II) as previously described [7]. Briefly, this mixture included ketamine (12.5 mg/kg; WDT, Garbsen, Germany), xylazine (2.5 mg/kg; Serumwerk Bernburg, Bernburg, Germany), and atropine (2.5 μg/kg; Braun, Melsungen, Germany) administered IM. In addition, 10 mL Ringer's solution warmed to body temperature was administered SQ prior to anesthesia. General anesthesia was induced and maintained with isoflurane (1.5 vol% in 100% O_2; CP-Pharma, Burgdorf, Germany), controlling the airways achieved through orotracheal intubation (size 2.0 uncuffed endotracheal tube) after laryngeal administration of lidocaine (Xylocaine pump spray; AstraZeneca, Wedel, Germany) in 8 animals. Two animals were maintained on inhalation anesthesia through a face mask because orotracheal intubation was unsuccessful. The mean total anesthesia time was 30 minutes.

2.4 | Gastrointestinal endoscopy

The animals were placed in left-lateral recumbency. A 3.8-mm flexible endoscope with a 1.2-mm diameter working channel (FUJI

EB-530P pediatric video bronchoscope, Düsseldorf, Germany) was used for esophagogastroduodenoscopy and rectocolonoscopy performed by a board-certified small animal internal medicine specialist.

2.4.1 | Esophagogastroduodenoscopy

Small bandage pieces aided to keep the mouth open and prevent damage to the endoscope by the marmosets' teeth (Figure 1A–B). Mild lubrication was applied to the endoscope, which was inserted through the larynx under visualization (Figure 1C–D). Insufflation and flushing during advancement of the endoscope through the esophagus and the gastric pylorus were performed each via a 5 mL syringe, and small amounts of air (0.5 mL) or sterile saline (0.5 mL) were repeatedly insufflated through the working channel for a total of 3–5 mL each. The mucosa in each segment was carefully inspected (Figure 2A–E). A total of 3 tissue biopsies were obtained from each gastrointestinal segment evaluated (stomach and

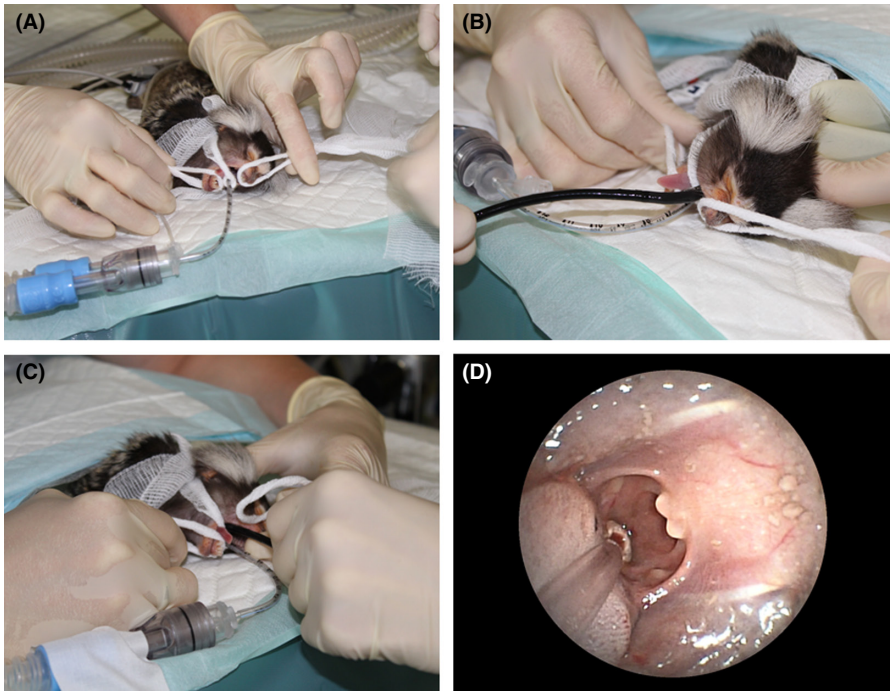

FIGURE 1 Preparation for esophagogastroduodenoscopy. (A, B) The mouth was kept open using small bandages, and (C, D) the endoscope was inserted through the larynx under visualization

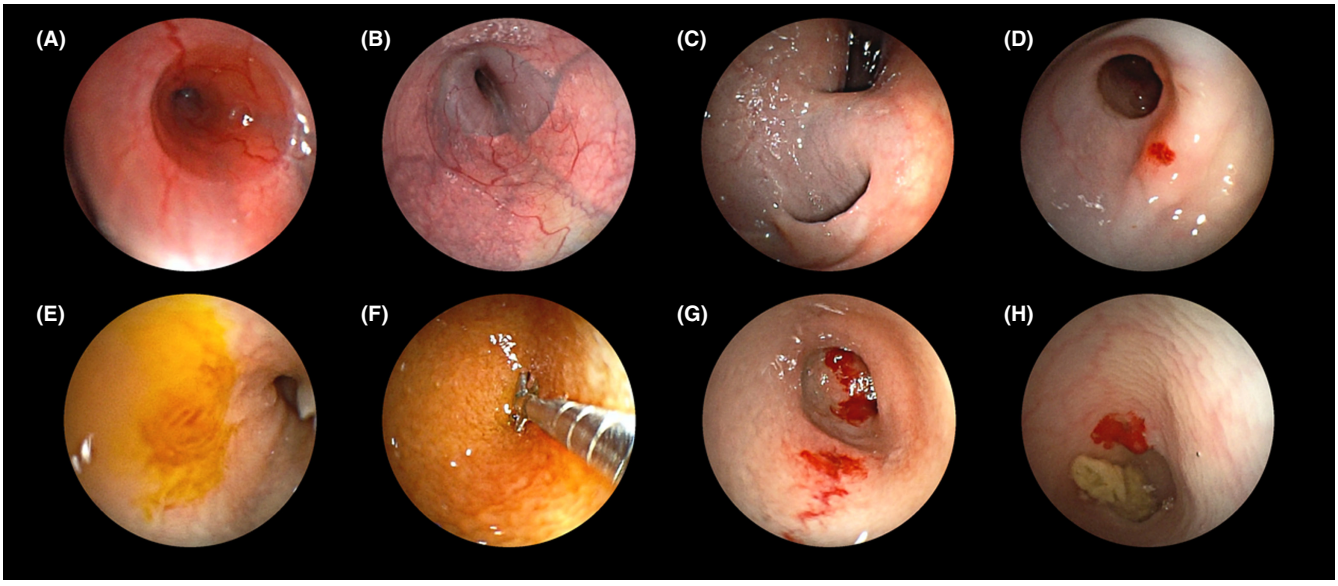

FIGURE 2 Esophagogastroduodenoscopy. Endoscopic view of the mucosa in the (A) proximal esophagus, (B) distal esophagus, (C) area of the lesser curvature of the stomach, (D) gastric pylorus, and (E) duodenum in the area of the papilla (covered with bile). (F) Endoscopic tissue biopsy (duodenum) was obtained, resulting in minor bleeding at the biopsy sites (G–H)

duodenum/proximal jejunum) using a flexible clamshell biopsy forceps (FTE biopsy forceps, short-oval spoon-shaped, mouth with window, flat ground coil, distal tapered, 120 cm; jaw diameter 1.0 mm, length 3.0 mm) through the working channel of the endoscope and at 90° against the mucosa (Figure 2F). Tissue biopsy samples from each segment were placed on separate small sponges kept moist with sterile saline (Figure 3) until immersed and fixed in 4% neutral-buffered formalin.

2.4.2 | Rectocolonoscopy

The animal was kept in left-lateral recumbency, and after application of mild lubrication, the endoscope was inserted through the anus under visualization (Figure 4A). Insufflation and flushing during advancement of the endoscope were performed as described. Colonic and rectal mucosa was carefully inspected (Figure 4B), and 4 colonic tissue biopsies (from the proximal, middle, and distal portion of the colon) were obtained. Ileal and cecal intubation was not attempted as the proximal colonic flexure (Figure 4C) could not reliably and safely be passed with the endoscope in most animals.

2.5 | Histopathology and special stains

Necropsy was performed following gastrointestinal endoscopy on 8 animals that were euthanized under general anesthesia (using GM II and 5% isoflurane followed by cardiocentesis after confirmed cardiac arrest) for medical ethical reasons (i.e., deteriorating clinical condition). Tissues were collected from several organs for further evaluation, and the gastrointestinal tract was removed and grossly examined (Figure 5A), evaluated for evidence of perforation from endoscopic biopsies (Figure 5B–D), and transmural (full-thickness) biopsies were obtained and placed in 4% neutral-buffered formalin.

Endoscopic and transmural gastrointestinal tissue biopsy specimens were embedded in paraffin, cut at 5 μm, and submitted for

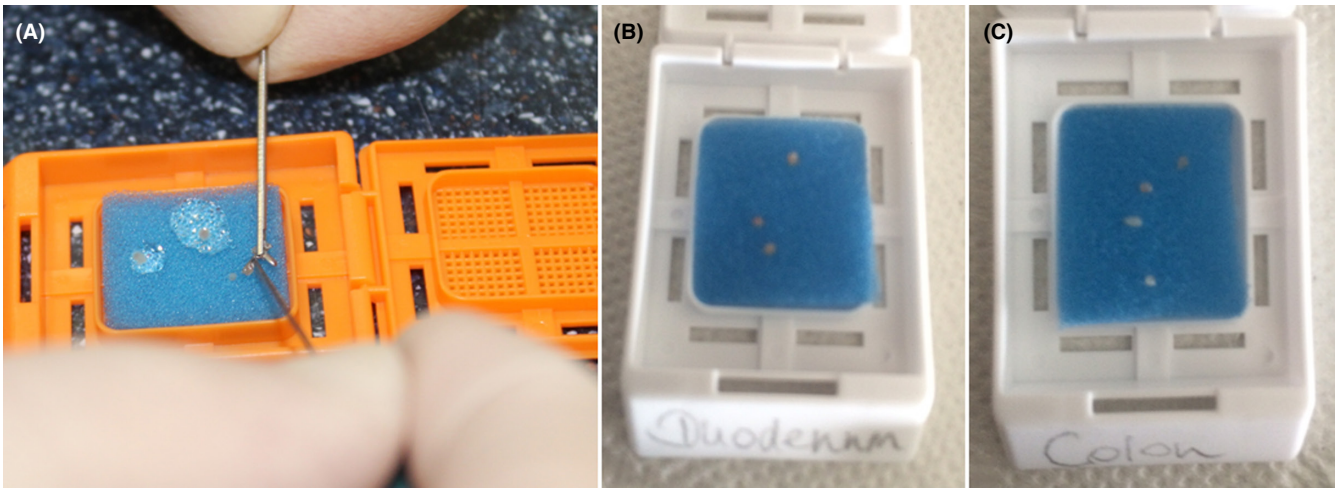

FIGURE 3 Endoscopic tissue biopsies. (A) Tissue biopsy samples were placed on small foam pads and were kept moist with sterile saline. (B, C) A total of 3–4 tissue biopsies were obtained from each segment of the gastrointestinal tract

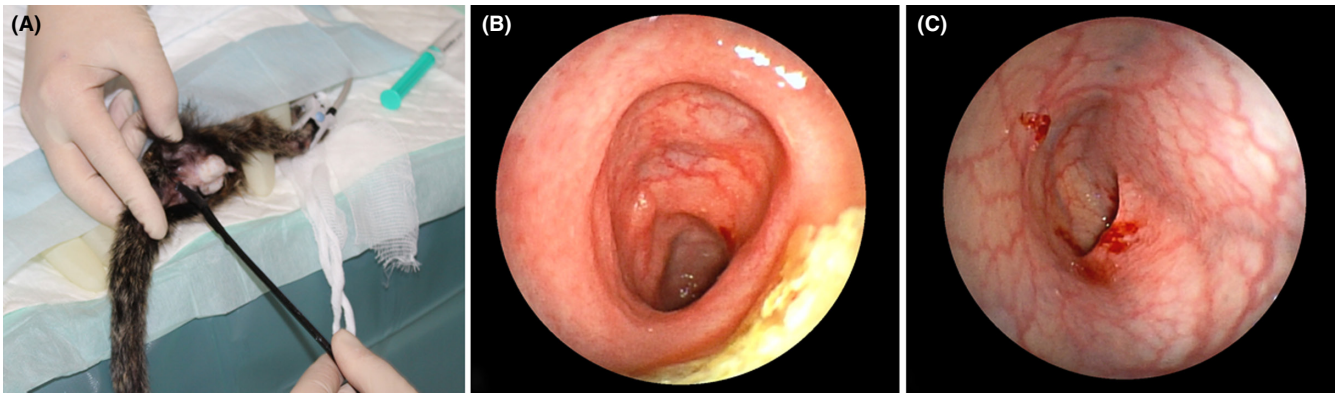

FIGURE 4 Rectocolonoscopy. (A) With the animal placed in left-lateral recumbency, the endoscope was inserted through the anus under visualization. (B) The colonic mucosa was carefully evaluated. (C) Narrowing at the proximal colonic flexure prevented successful endoscopic intubation of the ileum and cecum

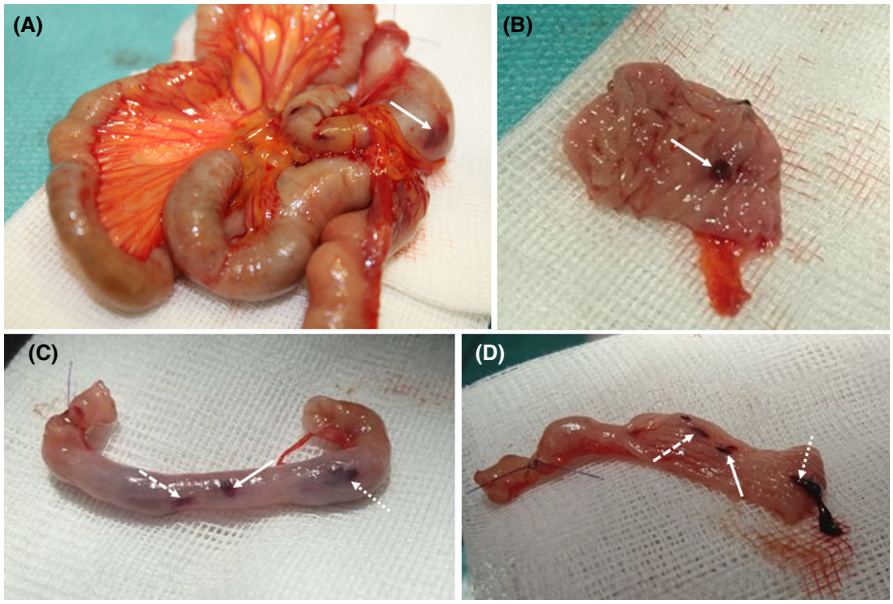

FIGURE 5 Necropsy evaluation. Following careful examination of the abdomen, (A) the gastrointestinal tract was removed, and (B–D) each segment was evaluated for evidence of perforation at the endoscopic biopsy sites (white arrows; A–B: stomach, C–D: duodenum)

routine histopathologic evaluation. Biopsy quality and diagnostic utility (i.e., size, mucosal layers included, tissue orientation, and presence of crush artifacts) were assessed, and the histopathologic findings were compared between both types of tissue biopsies (i.e., endoscopic specimens with the corresponding full-thickness biopsies).

3 | RESULTS AND DISCUSSION

Esophagogastroduodenoscopy was successful in all but one female marmoset, where intubation of the duodenum was not possible due to a presumed pyloric stricture. Marked proximal duodenal dilation resulting from pathologic flexion of the proximal (inferior) duodenal flexure could be an alternative explanation but is not typically associated with stricture formation [13]. Resulting from the narrowed gastric outflow tract or proximal duodenum, the stomach in this animal was affected by severe pre-stenotic dilation (Figure 6). In addition, endoscopic biopsies from the stomach, duodenum, and colon were successfully obtained from all marmosets, except for that one duodenum that could not be intubated. Blind biopsies (i.e., intubation of the duodenum with the biopsy instrument only and collection of biopsies without endoscopic visualization) were not attempted, given the risks associated with this technique [18].

All biopsies obtained from the gastrointestinal tract, despite being small in size (1 × 3 mm), were of adequate quality for histologic evaluation and lesion identification (Figure 7). Similar to conventional endoscopic tissue sampling in dogs and cats, the submucosal layer was reached in most specimens obtained from marmosets. Rectocolonoscopy was successful in all animals in this study, but the size of the marmosets remained a limiting factor for intubation and biopsy of the distal small intestine (i.e., ileum). Of importance, most endoscopic biopsy sites along the gastrointestinal tract were

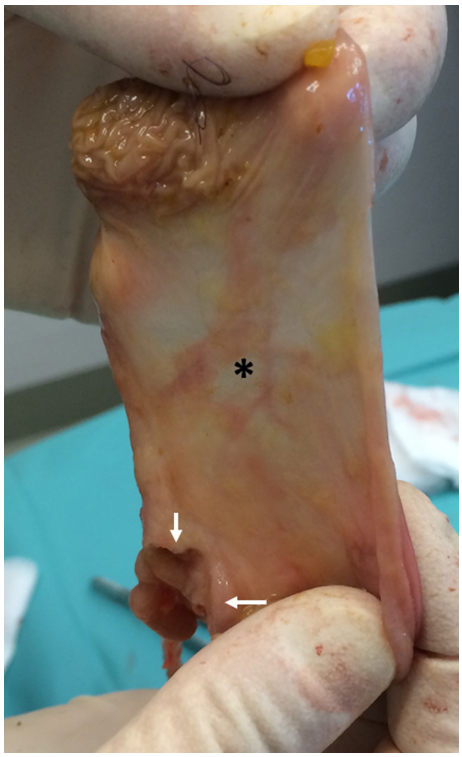

FIGURE 6 Gross pathology image of a presumed pyloric stricture. Esophagogastroscopy was successful in this female marmoset, but the duodenum could not be intubated due to pyloric narrowness caused by a pyloric stricture (indicated by white arrows). Note the marked pre-stenotic gastric dilation with thinning of the gastric wall (asterisk). A less likely explanation for this finding is marked proximal duodenal dilation resulting from pathologic flexion of the proximal (inferior) duodenal flexure

detectable by hemorrhage seen at the serosal surface. However, none of the biopsies had caused any perforation or other peri-procedural complications. Post-procedural complications were

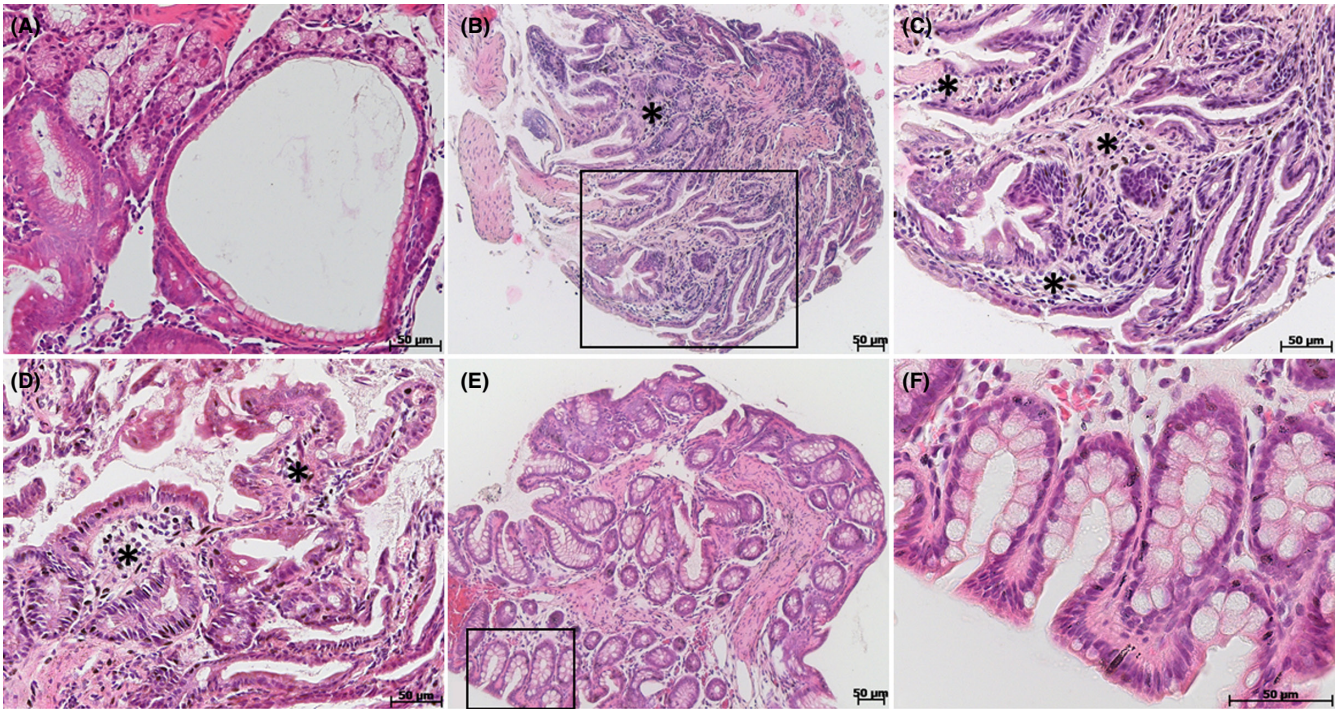

FIGURE 7 Histopathology images (H/E stain) of endoscopic tissue biopsies obtained from marmosets with chronic gastrointestinal signs. (A) Endoscopic biopsy from the stomach of a male marmoset, (B, D) endoscopic biopsies from the duodenum of a female marmoset, (C) larger magnification of the black insert in (B) showing leukocyte infiltration of the lamina propria (black asterisks), (E) endoscopic biopsy obtained from the colon of a male marmoset, (F) larger magnification of the black insert in (E)

also not noted in any of those animals recovered from anesthesia, including the male marmoset that underwent repeat diagnostic endoscopy more than 1 year after the first diagnostic procedure. Recovery from general anesthesia was fast (max. 2 hours), and voluntary food intake started within 6–10 hours. Bleeding at the endoscopic biopsy sites was minimal and ceased already during the endoscopic procedure, with no melena, hematochezia, or occult blood detected following recovery. However, marmosets recovered after combined esophagogastroduodenoscopy and rectocolonoscopy were clinically less severely affected than those humanely euthanized animals.

Maintenance of general anesthesia with isoflurane following orotracheal intubation is safe and efficient in marmoset patients and allows for prompt adjustment in the inhalant dose depending upon the patient's requirements. Laryngeal administration of lidocaine spray immediately prior to intubation appears to be important for preventing laryngeal edema and spasms compromising airway function.

In conclusion, gastrointestinal endoscopy comprised of esophagogastroduodenoscopy and rectocolonoscopy under general anesthesia, in the hands of an experienced endoscopist (i.e., at least some training and experience with gastrointestinal endoscopies), is a feasible and efficient technique to minimally invasively examine the gastrointestinal tract in marmosets and obtain a timely antemortem histologic diagnosis. Safely and efficiently obtaining diagnostic endoscopic small intestinal biopsies that allow for tissue analyses, as described in this report, improves the diagnostic armamentarium of clinicians (and researchers) handling common marmosets—particularly animals with chronic gastrointestinal signs—in captivity. These results provide an important avenue for further diagnostic evaluation and timely tissue diagnosis of marmosets affected by chronic gastrointestinal signs due to MWS or other chronic gastrointestinal conditions (e.g., lymphoma). Future investigations into the pathogenesis of chronic gastrointestinal diseases in marmosets, including gluten sensitivity and MWS, will benefit from the data presented. The ideal number of endoscopic biopsies to be collected from each gastrointestinal segment as a representative sample [19] remains to be determined in future clinical investigations. Future technical advances might allow for minimally invasive strategies in marmosets to evaluate segments of the gastrointestinal tract that are not within reach of currently available pediatric flexible endoscopes (i.e., ileum and large parts of the jejunum).

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests related to the publication of this manuscript.

DATA AVAILABILITY STATEMENT

Data and further information is available from the corresponding author upon reasonable request.

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