Intestinal full-thickness needle-core biopsy via laparotomy is safe, rapid, and effective and less invasive than standard incisional biopsy in dogs and cats

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OBJECTIVE
To describe the intestinal full-thickness needle-core biopsy technique via abdominal laparotomy outcomes and compare the histopathological and immunohistochemical diagnosis with standard incisional intestinal biopsy technique in dogs and cats.

ANIMALS
3 dogs and 17 cats.

METHODS
Client-owned dogs and cats were prospectively enrolled if intestinal full-thickness biopsies were indicated for the diagnosis of diffuse chronic intestinal diseases following ultrasonography. The study period extended from June 2021 to December 2022. All animals underwent intestinal biopsies with both techniques (needle-core biopsy and standard incisional biopsy) via abdominal laparotomy. Data collected included clinical signs, biopsy collection times, complications, and histopathologic and immunohistochemical findings. A minimum follow-up of 14 days was required.

RESULTS
The main clinical sign at presentation was chronic vomiting (65%). Mean needle-core biopsy collection time (262 seconds) was significantly shorter than standard incisional biopsy collection time (599 seconds; \( P < .000001 \)). The incidence of minor complications was 10% (inflammation of the skin surgical site secondary to licking). One catastrophic complication occurred on a standard incisional biopsy site in 1 cat in a context of bile peritonitis (5% of all cases). There were no complications associated with the needle-core biopsy. All but 1 cat were discharged, with a median of 2 days (range, 1 to 4 days) after surgery. The diagnoses resulting from both techniques were 100% concordant for the distinction between inflammatory bowel disease and intestinal lymphoma via histopathology and immunohistochemistry.

CLINICAL RELEVANCE
Needle-core biopsy is safe, rapid, and effective and is less invasive than standard incisional biopsy.

Keywords: histopathology, immunohistochemistry, inflammatory bowel disease, intestinal biopsy, lymphoma

Intestinal biopsy is used to diagnose chronic intestinal diseases in dogs and cats (eg, inflammatory bowel disease [IBD], intestinal lymphoma [IL], lymphangiectasia, and metastatic disease).1,2 The major complications reported with the traditional standard incisional full-thickness biopsy technique include dehiscence of the enterotomy incision and septic peritonitis.3 Reported complication rates following incisional full-thickness small intestinal biopsies are up to 11% for minor postoperative complications and up to 12% for major postoperative complications between 3 and 9 days postoperatively.2-5 The majority of major complications result in mortality, primarily due to the reluctance of owners to repeat surgery and thus electing for euthanasia.4

Recent advances in sample collection techniques aim to reduce complications, such as endoscopic biopsies or ultrasound-guided cytology.6,7 Despite the associated risks, full-thickness biopsies via exploratory laparotomy are more informative than partial-thickness endoscopic biopsies. Endoscopic biopsies
do not provide sufficient information to differentiate small intestine diseases such as IBD and IL in cats. A surgical biopsy enables harvesting of a sample of every intestinal layer, while the laparotomy itself allows visualization and access to all abdominal organs, including full intestinal tract. Thus, endoscopy can result in incomplete, inconclusive, or incorrect histopathological results. Ultrasound-guided fine-needle cytological samplings of gastrointestinal lesions are technically challenging and are only clinically relevant in about two-thirds of cases. Alternative full-thickness biopsy methods have been developed. Keyes or dermatology punch biopsies are associated with equivalent surgical durations and complication rates to the standard full-thickness technique.

Needle-core biopsy instruments are commonly used for liver, spleen, kidney, and soft tissue and thoracic masses. Automated and semiautomated needle-core biopsy instruments can be used with direct visualization of the tissue or under ultrasound guidance.

To the authors’ knowledge, no studies have described the intestinal needle-core biopsy in dogs, cats, or other species. The objective of this study was to describe the intestinal full-thickness needle-core biopsy technique via abdominal laparotomy, report outcomes, and compare the histopathological and immunohistochemical diagnosis with the standard incisional intestinal biopsy technique in dogs and cats. We hypothesized that the needle-core biopsy technique would be a safe, rapid, and effective option for intestinal biopsy, with low complication and mortality rates in dogs and cats.

Methods

Case selection

Dogs and cats of all ages examined at the Centre Hospitalier Vétérinaire Atlantia, Nantes, France, from June 2021 to December 2022 with clinical signs and ultrasonography that warrant intestinal full-thickness biopsies for the diagnosis of diffuse chronic intestinal diseases were eligible for the study. Signalment (including age, breed, gender, neutering status, weight, and body condition score), preoperative clinical signs and duration, preoperative imaging results (ultrasonography and endoscopy when performed), laboratory findings (CBC, biochemistry, urinalysis), preoperative medications administered (in particular recent corticosteroid administration), and any concomitant disease (eg, diabetes, hyperthyroidism) were recorded. Preoperative blood analysis included albumin, total protein, and ALT measurements. A minimum follow-up of 14 days was required to ensure intestinal healing was achieved. Dogs and cats with an isolated intestinal mass were excluded from the study such that only animals with diffuse intestinal diseases were included. These animals were prospectively included in the clinical study and underwent intestinal biopsy with both techniques (needle-core biopsy and standard incisional biopsy) during the same anesthesia by qualified surgeons (European College of Veterinary Surgeons [ECVS] diplomate or ECVS residency trained). The study was approved by an ethics committee (CERVO-2021-9-V). Owner consent was obtained before surgery.

Anesthetic and analgesic protocol

The anesthetic protocol used in each animal varied slightly and was consistently validated by one of the anesthesiologists (European College of Veterinary Anaesthesia and Analgesia diplomate or resident under direct supervision of a European College of Veterinary Anaesthesia and Analgesia diplomate). Opioids (methadone hydrochloride, morphine sulfate, or fentanyl citrate) were consistently administered during premedication, induction, anesthesia in continuous rate infusion (CRI), or in combination. Ultrasound-guided transversus abdominis plane block was performed at the anesthesiologists’ discretion. Anesthesia was maintained with inhaled isoflurane and oxygen.

Patient preparation

All animals were clipped and aseptically prepared for abdominal surgery. They were placed in dorsal recumbency, and laparotomy was performed with consistent exploratory evaluation of the abdominal cavity. Every abnormality was recorded (eg, abdominal effusion, hepatic nodules). Each of the 3 sections of the small intestine (duodenum, jejunum, and ileum) was biopsied. A segment of approximately 5 to 10 cm was isolated and occluded by an assistant either digitally or using Doyen clamps.

Surgical procedure

Small intestinal biopsy with standard incisional biopsy technique—The surgeon made a 1-cm longitudinal antimesenteric incision in the middle of the isolated portion of intestine with a new No. 11 scalpel blade. A thin full-thickness biopsy was obtained from the cut edge with Metzenbaum scissors (Figure 1).

![Intraoperative picture after 1-cm standard incisional intestinal full-thickness biopsy and before closure of the enterotomy.](image)

Closure was performed with 4-0 USP taper point polydioxanone (Monotime; Peters Surgical) in a simple continuous longitudinal suture pattern. Biopsy collection time was measured from the start of the first incision to the end of closure (last knot of the simple continuous suture). The biopsy sample was immediately placed into formalin.

Small intestinal biopsy with a semiautomated needle-core biopsy instrument—After each standard
incisional biopsy, 2 needle-core biopsies were performed, 1 cm orally and 1 cm aborally from the standard biopsy site, via a new 16-gauge Tru-cut device (TEMNO [16 gauge X 9.0 cm]; Merit Medical Systems). The surgeon inserted the semiautomated device through the intestinal wall into the lumen of the intestine at an angle of approximately 45° to the antimesenteric surface. Caution was taken not to cross the mesentery at an angle of approximately 45° to the antimesenteric surface. The surgeon inserted the semiautomated device (TEMNO [16 gauge X 9.0 cm]; Merit Medical Systems). Standard biopsy site, via a new 16-gauge Tru-cut device into the lumen of the intestine. Postoperative care

All animals were hospitalized for a minimum of 2 days. Ultrasonography was performed 2 days postoperatively to detect any short-term dehiscence and repeated before discharge if indicated. Time to hospital discharge after surgery was noted.

Histopathological evaluations

Standard incisional biopsies from each portion of intestine were identified separately and sent to a board-certified pathologist (European College of Veterinary Pathologists diplomate). Identification included the name, surname, species, type of biopsy (“S” for “standard incisional biopsy”), and the portion of intestine (“D” for “duodenum,” “J” for “jejunum,” and “I” for “ileum”). Any biopsies of other organs were sent to the pathologist concurrently. Paired needle-core biopsies from each portion of intestine were identified separately and anonymously. Identification included an anonymous combination of numbers and letters, the species, the type of biopsy (“T” for “Tru-cut needle-core biopsy”), and the portion of the intestine (“D” for “duodenum,” “J” for “jejunum,” and “I” for “ileum”). They were sent to the pathologist at the end of the study. All biopsies were analyzed by a single European College of Veterinary Pathologists diplomate (JM) for histopathology and CD3+/CD20+ immunohistochemistry study. Histopathologic evaluation followed guidelines of the World Small Animal Veterinary Association Gastrointestinal Standardization Group (extended to full-thickness biopsy rather than endoscopic biopsy) and the WHO classification of lymphoid neoplasms.12,13

Follow-up

Two follow-ups (phone call, mail, or recheck) were made at approximately 5 and 14 days to record complications and clinical signs. In most cases the results of the histopathology analysis were available at the first follow-up appointment.

Complications

Postoperative complications were recorded. The complication was considered minor if it resolved without surgical intervention. The complication was considered major if resolution required surgical intervention. The complication was considered catastrophic if death occurred during the 14-day follow-up period.

Statistical analysis

All statistical analyses were performed with R, version 4.2.0 (R Foundation for Statistical Computing). Statistical significance was set at P < .05. Descriptive population statistics were reported as frequency (%) for categorical data and mean ± SD or median (range, minimum to maximum) for continuous data. Sample size was determined with the Fisher exact test (2 sided) assuming independence. This approach was used instead of the McNemar test because there was no way to determine expected frequency between each technique. The diagnostic yield of standard incisional biopsy (3 samples: duodenum, jejunum, and ileum) was estimated at 98%
for both species. A power analysis was performed and established that a sample size of 18 animals would provide ≥ 80% power to detect a decrease in diagnostic yield of 38%, with a 95% confidence level (2-sided Fisher exact test). Post hoc power analysis with 20 animals was calculated and was 86%. Continuous variables (biopsy collection times for both techniques) were assessed for normality and compared with the use of a paired test.

Results

Signalment

Three dogs and 17 cats that underwent intestinal biopsy met the inclusion criteria. All 3 dogs were intact males. Eight cats were neutered males and 9 were spayed females. Represented breeds were domestic shorthair (n = 16), and 1 each of Pug, Korat, Finnish Lapphund, and Miniature Pinscher. The mean age was 10.1 ± 3.6 years, and the mean body weight was 4.3 ± 1.9 kg. The mean 9-point body condition score was 4.1 ± 1.7.

Clinical signs and pathology

Clinical signs at presentation included chronic vomiting (65%), lethargy (55%), anorexia or decreased appetite (50%), weight loss (50%), chronic diarrhea (25%), and other (35%). Other clinical signs included hyperthermia (n = 2), dehydration (1), constipation (1), expiratory dyspnea (1), coughing (1), and jaundice (1). The duration of clinical signs ranged from 10 days to 3 years. Only 3 of the 20 animals received steroids within the 15 days prior to surgery. Steroids were given for 7 days to animal number 7 and stopped 1 day before surgery (prednisolone [0.5 mg/kg, PO, q 12 h]); for 3 weeks to animal No. 14 and stopped 5 days before surgery (prednisolone [0.5 mg/kg, PO, q 24 h]); and for 2 weeks to animal number 20 and was not stopped before surgery (prednisolone [0.5 mg/kg, PO, q 24 h]). Median albumin level was 26.5 g/L (range, 19.0 to 33.0 g/L). Five animals (25%) were hypoalbuminemic (< 25 g/L). Median total protein level was 67.5 g/L (range, 3.90 to 98.0 g/L). Five animals (25%) were hypoproteinemic (< 60 g/L). Median ALT activity was 37.0 IU/L (range, 11.0 to 209.0 IU/L). Five animals (25%) presented with high ALT activity. Two cats (animals No. 8 and 20) were known as positive for FIV. Fourteen animals (70%) suffered at least 1 concurrent disease (eg, chronic kidney disease, bladder stones).

Diagnostic imaging

All animals underwent abdominal ultrasonography prior to surgery. All animals showed diffuse parietal thickening of the small intestine.

Surgical procedure

All animals received antibiotics (amoxicillin, ampicillin-sulbactam, or cefazolin depending on availability) approximately 30 minutes prior to skin incision and every 90 minutes thereafter while the animal was under general anesthesia. Mean total biopsy collection time for standard incisional biopsy was 599 ± 253 seconds (range, 325 to 1,230 seconds). Mean total biopsy collection time for needle-core biopsy was 262 ± 75 seconds (range, 185 to 427 seconds). Mean total biopsy collection time was significantly shorter for needle-core biopsy than for standard incisional biopsy (P < .000001). Omental wrapping and complete abdominal lavage were performed in 7 (35%) and 4 (20%) cases respectively (including 1 cat with preexisting biliary peritonitis). Other biopsies included mesenteric lymph nodes (n = 17), pancreas (5), liver (5), and stomach (2). Concurrent surgical procedures were performed in 1 cat (cholecystectomy in a context of preexisting biliary peritonitis). The gallbladder was sent for histopathological examination. Esophagostomy feeding tubes were placed during the same anesthetic period in 7 cats and none of the dogs, at the surgeons’ discretion.

Intraoperative complications

No intraoperative complications occurred in the animals.

Hospitalization

During hospitalization, analgesia was provided by opioids (CRI of fentanyl-ketamine, CRI of fentanyl-lidocaine-ketamine, morphine, methadone, and butorphanol according to pain scores). All animals received antibiotics during hospitalization. Other postoperative treatments varied according to each case.

Ultrasoundographic check-up

Repeat ultrasonographic examination was performed in all but 1 cat because of behavioral problems during hospitalization (animal No. 4). The final postoperative ultrasound examination during hospitalization was performed at a median time of 2 days after surgery (range, 2 to 4 days). Ultrasound did not reveal any postoperative abnormalities in 18 of the 19 (95%) animals examined and were thus discharged. The examination revealed signs of septic peritonitis in 1 animal (No. 20), necessitating a second surgery.

Discharge

All but 1 of the cats were discharged from the hospital. Median hospitalization time was 2 days after surgery (range, 1 to 4 days). One cat was discharged at 1 day after the operation because of behavioral problems during hospitalization.

Postoperative complications and outcome

Mild inflammation of the surgical skin site secondary to ligation occurred in 2 cats at day 5 and day 7 postoperatively and was classified as a minor complication. The minor complication rate was 10%. These minor complications were related to the laparotomy itself, not the intestinal biopsy. One cat died secondary to septic peritonitis on day 4 postoperatively despite a second surgery with enterectomy on the day of diagnosis (animal No. 20 with marked IBD). The cat presented with an intestinal suture dehiscence (Figure 3) on the standard incisional jejunal biopsy site. This animal was the only animal that underwent intestinal biopsies in the context of preexisting peritonitis (bile peritonitis) and that underwent a concurrent surgical procedure (cholecystectomy).
The histopathological study of core biopsies presented adequate diagnostic quality.

Histopathological findings

- **Histopathological findings with standard incisional biopsy technique**—The histopathological study of standard incisional biopsies revealed IBD (n = 16) and IL (5), with 1 cat (animal No. 2) presenting both patterns depending on the intestinal site. When IBD was present, infiltration was classified as lymphoplasmacytic (n = 16), eosinophilic (11), neutrophilic (4), and other (macrophage [1]). The severity of this IBD was graded as mild (n = 1), moderate (3), and marked (12).

- **Histopathological findings with needle-core biopsy technique**—The histopathological study of needle-core biopsies revealed IBD (n = 16) and IL (5), with 1 cat presenting both patterns depending on the intestinal site (animal No. 2). When IBD was present, infiltration was classified as lymphoplasmacytic (n = 16), eosinophilic (12), neutrophilic (2), and other (0). Severity of this IBD was graded as mild (n = 0), moderate (2), and marked (14).

- **Histopathological agreement**—Total agreement (100%) was seen between biopsies from both techniques concerning distinction between IBD and IL. When IBD was present, agreement from both techniques in cases of lymphoplasmacytic, eosinophilic, and neutrophilic infiltration was 100%, 95%, and 90%, respectively.

- **Other histopathological findings**—In 5 standard incisional ileal biopsies, there was insufficient mucosa for evaluation. All other standard incisional ileal biopsies, all standard duodenal and jejunal biopsies, and all needle-core biopsies presented adequate diagnostic quality.

**CD3+/CD20+ immunohistochemistry study**

The CD3+/CD20+ immunohistochemistry study of the standard incisional biopsies revealed a mixed heterogeneous CD3+/CD20+ infiltrate consistent with IBD in 16 cases and a massive CD3+ infiltrate consistent with IL in 5 cases. The same study was performed on needle-core biopsies and revealed the same distribution. Total agreement (100%) was seen between biopsies from both techniques concerning the immunohistochemical differentiation of IBD and IL.

**Agreement between histopathological and CD3+/CD20+ immunohistochemistry findings**

There was 100% agreement between histopathological and CD3+/CD20+ immunohistochemistry findings concerning distinction between IBD and IL.

**Final follow-up**

After the second follow-up, 1 dog (animal No. 1 with marked IBD) and 1 cat (animal No. 15 with IL) were euthanized respectively at 55 and 46 days postoperatively. The dog suffered from deterioration of gastrointestinal signs despite treatments. The cat had respiratory distress and was in shock. The final follow-up for the other animals was at a median time of 23 days (range, 14 to 406 days).

**Discussion**

In the present study, in which 20 animals underwent intestinal biopsies, results indicated that the needle-core biopsy technique was safe, rapid, and effective and was less invasive than the standard incisional biopsy technique, with low morbidity and mortality rates and a very good agreement.

The complication rate was low despite both biopsy techniques (needle-core biopsy and standard incisional biopsy) being performed on the same patients. Biopsies were performed by qualified surgeons (ECVS diplomate or ECVS residency trained) who were aware of both techniques, and this fact could have had a positive impact on limiting postoperative complications. Esophageal tube placement in 7 cats could have reduced the complication rate in helping early postoperative feeding. In previous studies,1,4,15 hypoalbuminemia was identified as a risk factor for enterotomy dehiscence. In the present study, 25% of animals were hypoalbuminemic versus 34% in the 2005 study by Shales et al,4 with a higher complication rate. The low complication rate in our study could thus be due to a relatively low incidence of hypoalbuminemia or the smaller sample size.

As both biopsy techniques were performed on the same patient at a relatively small distance, and as the needle-core biopsies were studied anonymously, each animal was considered as its own control group. Diagnostic agreement between biopsies from both techniques has been validated. Needle-core biopsies appeared consequently clinically relevant.

In the current study, to avoid multiplying biopsies and the associated risks, each portion of the intestine was biopsied 3 times: 1 standard incisional
biopsy and 2 needle-core biopsies. The authors chose to perform 2 needle-core biopsies at each site to be as accurate as the standard incisional biopsy site and to avoid missing the lesion. One needle-core biopsy was located just orally from the standard incisional biopsy site. The other one was located just aborally from the standard incisional biopsy site. As the needle-core biopsies were small, performing 2 biopsies seemed reasonable. In total, 3 standard incisional biopsies and 6 needle-core biopsies were made. The 3 standard incisional biopsies and the 6 needle-core biopsies separately seemed sufficient for diagnosis. However, if only needle-core biopsies were performed, more could be safely used if deemed appropriate. This could enable more precise sampling and also provide a more representative picture of the entire intestine. Further studies are warranted to determine the most appropriate number of needle-core biopsies to collect.

Following histopathology, CD3+/CD20+ immunohistochemistry was used to improve diagnostic accuracy. To further improve accuracy, clonality analysis with PCR could have been used, but we chose not to use this in the current study.\textsuperscript{16}

We chose to insert the needle-core device at an angle of approximately 45° angle for 2 reasons. First, subjectively, this reduces the risk of accidental puncture of the mesenteric aspect of the intestine in the authors’ experience. Second, the authors hypothesized that oblique insertion would reduce the risk of leakage by improving the immediate sealing after suture. However, there have been no experimental studies to measure intraluminal pressure during leak testing to confirm this, or any histopathological study of the intestine during healing. The authors recommend maintaining the inner stylet of the needle-core device in close contact with the intestinal wall during the biopsy shoot to ensure good sample collection. This reduces the risk of failing to sample any intestinal tissue; it was also deemed important to consistently check the sample in the needle-core device before closing the intestine (Figure 4).

Biopsy collection time was significantly faster with the needle-core technique (262 seconds) than with the standard incisional technique (599 seconds; \( P < .000001 \)). A needle-core biopsy site requires only a single simple interrupted pattern for closure, whereas a standard incisional biopsy site requires a simple continuous longitudinal suture pattern for closure. There is an inherent time advantage of the needle-core biopsy technique during closure. A simple interrupted pattern is also more secure than a simple continuous longitudinal suture pattern.

The needle-core technique is easy to acquire and perform. Veterinarians with basic skills in intestinal surgery can safely learn how to use the device. When biopsies are performed, the small intestine can be very thickened with abnormal intestinal layers impairing normal healing. Appositional sutures can be difficult to perform, predisposing to early intestinal leakage. A major advantage of performing a tiny needle-core biopsy in these rigid intestines is the simplicity of closure compared to a larger standard incisional biopsy. The intestine is also less prone to leakage when the biopsy site is smaller.

The length of the needle-core device used was 9.0 cm. Other lengths are available, but the authors do not recommend longer devices, as they are less handy. Shorter ones may be easier to use, but they were not available at our practice at the time of the study. Other diameters also exist, but only 16-gauge diameter was used in the study. This diameter seems to fit for all sizes of cats and dogs. The device is not prohibitively expensive (approx $33), but it costs more than a scalpel blade (< $1.10). The device was reused between intestinal sites of the same animal, as for the scalpel blade. The risk of tumor cell dissemination or bacterial contamination is probably unchanged between techniques. The needle-core technique can be recommended for small animal practitioners. However, the authors recommend keeping the animal in hospitalization for a minimum of 2 days to monitor for potential intestinal leakage. The cost of performing laparotomy biopsies and hospitalization is, however, often greater than the cost of performing endoscopic biopsies. This minimally invasive technique could also be used under laparoscopic or laparoscopy-assisted abdominal exploration. Further studies are warranted.

Our study has several limitations. First, the number of cases was reduced for ethical reasons (to assess both techniques on a minimal number of animals). More cats were included because there are probably more prone to or more severely affected by diffuse chronic intestinal diseases than dogs and because of our recruitment (prospective study). Second, clonality analysis with PCR could have been used to improve the accuracy of the diagnosis. This test was not routinely available in the practice’s histopathology laboratory, and additional costs would have been incurred. Third, as the aim of the study was to prospectively describe the safety and effectiveness of the intestinal full-thickness needle-core biopsy technique, there was no intention to focus on long-term follow-up. Finally, as there was a low number of animals and complications, no preoperative risk factor was identified. Since the method of biopsy has proven to be safe and effective, more clinical cases can be enrolled for future clinical studies.

The needle-core biopsy technique was safe for intestinal biopsy via abdominal laparotomy. There were no complications associated with this biopsy.
technique in any of the 20 animals enrolled in the study. There was complete agreement (100%) between needle-core biopsy and standard incisional biopsy for histopathological and immunohistochemical diagnosis for the distinction between IBD and IL and very good agreement (90-100%) for type of infiltration in cases of IBD.

Needle-core intestinal biopsy via abdominal laparotomy can be considered to be a safe, rapid, and informative alternative to standard incisional biopsy.

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