Diagnostic yield of percutaneous, ultrasound-guided, fine needle aspirates of the gastrointestinal wall: a retrospective analysis of 152 samples

ORIGINAL ARTICLE

Diagnostic yield of percutaneous, ultrasound-guided, fine needle aspirates of the gastrointestinal wall: a retrospective analysis of 152 samples

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Objectives: The aim was to assess the technical success of percutaneous ultrasound-guided fine needle aspirates of gastrointestinal wall lesions and evaluate predictors of success. Secondary aims included comparing the cytological diagnosis with histopathology, evaluating the utility of concurrent locoregional lymph node cytology and assessing the procedure's complication rate.

Material and Methods: Gastrointestinal wall cytology from 75 dogs and 70 cats obtained between 2018 and 2023 were reviewed and categorised as successful (resulting in a diagnostic cytology report) and accurate (resulting in the correct diagnosis when compared to histopathology). Unsuccessful fine needle aspirates, not submitted for cytology, were not recorded. Variables recorded included animal signalment, lesion and lymph node's appearance on ultrasound, size, location, number of smears submitted and experience of the ultrasonographer.

Results: One hundred and fifty-two reports were analysed. Eighty-eight (58%) were successful: three normal epithelium, 21 inflammatory processes and 64 neoplasms. Variables associated with increased technical success included description of a mass, higher number of slides submitted and thickness of gastrointestinal lesion on ultrasound. Comparison with histopathology, performed for 17 lesions, showed discrepancies in eight, complete agreement in seven and partial in two. Eighty-four locoregional lymph nodes were sampled, of which, 67 were successful (80%) and 52 brought additional clinical information (supporting GI wall cytology or diagnosing neoplasia not identified on GI wall cytology). No complication strictly attributable to gastrointestinal wall sampling was reported but when possibly related, death of the patient occurred in 2.5% of cases.

Clinical Significance: Ultrasound-guided fine needle aspirate of gastrointestinal wall had moderate accuracy and was unsuccessful in 42% of cases, but technical success increased when sampling mass lesions, thicker intestinal layers and submitting more slides.

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INTRODUCTION

Gastrointestinal (GI) neoplasms account for up to 11.4% and 11.9% of all canine and feline tumours respectively (Grüntzig et al., 2015; Rissetto et al., 2011). Whilst abdominal radiographs are diagnostically important in acute GI disease, ultrasonography is the GI imaging modality of choice for assessing intestinal layering, including the presence of masses (Albury, 2015; Meomartino et al., 2021; Seiler et al., 2022; Simeoni et al., 2020; Tyrrell & Beck, 2006; Won et al., 2015). It also enables appraisal and measurement of locoregional lymph nodes (LLNs) which has utility in discriminating feline enteritis and low-grade intestinal lymphoma (Freiche et al., 2021). Ultrasonography lacks specificity to discriminate between benign and malignant intra-abdominal lesions (De Swarte et al., 2011; Millar & Zersen, 2021; Pecceu et al., 2020). Therefore, with GI lesions, sampling is required to determine their nature. Whilst histopathology remains the diagnostic gold standard, cytology of ultrasound-guided fine needle aspirates (FNAs) offers many benefits, including minimal invasiveness, affordability, feasibility under sedation and fast turnaround (Bonfanti et al., 2006; Skeldon & Dewhurst, 2009; Wypij, 2011).

Although reported as relatively safe, FNAs of the GI tract remain contentious among clinicians (Léveillé et al., 1993; Vignoli & Saunders, 2011). The procedure is often omitted due to risk of GI wall perforation and secondary septic peritonitis, or because cytology is believed to be unrewarding (Wypij, 2011). Variables that impact on cytological diagnosis relate to the lesion itself (nature, accessibility and size), the operator's experience (ultrasound and microscopy) or the technique (needle size, aspiration versus strict insertion, number of passes, number of smears, spreading) (Arai et al., 2019; Liffman & Courtman, 2017; Llanos et al., 2023; Sapierzyński et al., 2017; Stone, 1995; Whitlock et al., 2021; Wypij, 2011).

Literature on the diagnostic yield and safety of GI tract FNAs is limited (Bonfanti et al., 2004, 2006; Crystal et al., 1993; Turner et al., 2021). Two studies specifically investigated the diagnostic value of cytology in confirmed GI neoplasia ($n = 67$) and mass lesions ($n = 13$). However, both the presence of a mass lesion on ultrasound and the diagnosis of neoplasia are factors that could positively influence cytological yield (Bonfanti et al., 2004, 2006). The clinical usefulness of percutaneous ultrasound-guided FNAs of GI lesions has only been described by Crystal et al., 1993 ($n = 12$) and Turner et al., 2021 ($n = 44$). Both report moderate technical success (68 to 75%) and moderate to high diagnostic accuracy when compared to histopathology (67% to 82%) (Crystal et al., 1993; Turner et al., 2021). The development of complications was not specifically assessed in previous studies on GI wall FNAs but the absence of complication was reported in most (Bigge et al., 2001; Bonfanti et al., 2004; Crystal et al., 1993; Turner et al., 2021; Wypij, 2011).

The aim of this study was to retrospectively assess the technical success (FNA resulting in a cytology report with tentative identification of the underlying process) of percutaneous, ultrasound-guided GI wall FNAs as well as assessing lesion and technical variables as predictors of success. Secondary aims included the evaluation of diagnostic accuracy (FNA resulting in the correct diagnostic when compared to histopathology), appraisal of the benefits of sampling the LLN and quantification of complications after the procedure. We hypothesised that GI wall FNAs would have moderate technical success, high diagnostic accuracy and low complication rate.

MATERIALS AND METHODS

Cytology reports, appraised by a board-certified clinicopathologist or resident under their supervision, of dogs and cats that had percutaneous ultrasound-guided FNAs of the GI tract performed in our institution between December 2018 and July 2023 were retrospectively reviewed by the same author (PH). The cytology database was interrogated with key words (GI, gastric, intestinal, duodenal/duodenum, jejunal/jejunum, ileal/ileum, caecal/caecum, colic/colon, wall, thicken, layer, eosinophilic sclerosing fibroplasia or with the combination of gastric/intestinal and mass, lesion, cancer/neoplasia/neoplasm, carcinoma/adenocarcinoma, lymphoma, sarcoma, leiomyoma/sarcoma, mast cell, round cell, spindle cell and mesenchymal cell). Cases were excluded if FNAs were not obtained percutaneously with ultrasound guidance or if the location of the lesion could not be determined from the imaging report or medical file. Cases undergoing GI wall FNA for which the procedure was unsuccessful (no yield) and did not result in a cytology submission could not be accounted for as this information is not recorded at our institution.

Each cytology report was classified as either technically successful (FNA yielding enough cells to result in a cytology report with tentative identification of the underlying process) or unsuccessful (non-diagnostic samples, as per clinical pathologist's description including reports of insufficient cellularity or preservation). Successful samples were further categorised as normal, inflammatory (including reactive processes) or neoplastic. The number of smears submitted was recorded.

Categorical and continuous variables were recorded as potential factors of success. The animal signalment (species, body weight, body condition score, sex and reproductive status) was extracted from the medical file. Further factors considered included location of the lesion (stomach, duodenum, jejunum, ileocolic and colon), ultrasonographic appearance including, alteration of the layering, thickness of the GI lesion and presence/absence of a mass lesion were recorded as stated in the imaging report. When information on the aspect of the lesion (size, preservation of the layering and echogenicity) was not clearly stated on the imaging report or readily available on stored images (callipers), images were reviewed by a resident in diagnostic imaging (RR). Given the absence of guidelines on what constitutes a GI mass on ultrasound; in this study, a mass was considered present when described subjectively by the ultrasonographer

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in the imaging report and, objectively, when the wall thickness was equal or greater to 15 mm (Penninck et al., 2003) or 20 mm (Table 3, Turner et al., 2021). For LLN, location, size, hypoechogenicity and homogeneity were recorded.

Experience of the ultrasonographer (intern, resident or board-eligible; all under boarded specialist supervision) was retrieved from the imaging report. The evaluation of the detailed technique of the FNA was not recorded (size of the needle, number of passes, aspiration or not) and for this reason, only the number of slides submitted was extracted.

Concordance of the cytological results between GI lesion and LLN was evaluated when available. Sampling of the lymph node was considered to add clinicopathological information when lymph node cytology confirmed the GI wall diagnosis (i.e., normal, inflammatory or neoplastic), when cytology of the LLN was of diagnostic quality and did not suggest metastasis of a primary GI neoplasia (normal or inflammatory cytology in face of diagnosed neoplasia on GI wall FNAs), and when sampling of the GI lesion was not diagnostic but FNAs of the lymph node diagnosed neoplasia.

When available, medical files were reviewed for subsequent histopathology and for the development of complications. The diagnostic accuracy of the GI wall FNAs was based on comparison of the cytology results with histopathology. Agreement was considered as "complete" when the disease process and cell lineage were identical, as "partial" when the disease process was similar, but the cell lineage could not be determined on cytology. A "lack of agreement" represented a discrepancy of the main pathological process. The final diagnosis was based on histopathology when available or cytology (from the GI wall and/or lymph node) otherwise; except in a single case of discrepancy where cytology diagnosed neoplasia and histopathology from endoscopy biopsies returned inflammation.

Complications were categorised as present, possible or absent. Patients euthanased because of a poor prognosis within 48 hours of FNA sampling were excluded from complication-based assessment. Complications were present in patients developing either significant haemorrhage within 24 hours of sampling or septic peritonitis within 7 days of discharge (Bigge et al., 2001; Reece et al., 2020). Patients euthanased within 4 days of FNA sampling without a cause of death available were classified as possible complications. Absence of complication was based on survival to discharge and absence of reported complications. When available, follow-up (in days) based on subsequent revisit or recorded contacts with owners or referring veterinarians was extracted from medical records. Otherwise, the method for assessing complications prior to discharge was recorded (physical examination and discharge on the day, overnight hospitalisation and/or point of care ultrasound of the abdomen).

Data were organised in Microsoft Excel (Microsoft, USA) and statistical analyses performed in R (R version 4.3.0) using RStudio (version 2023.9.1.494). Normality was assessed by visual inspection of data. Chi-square test was used to compare independent categorical data (species, sex, reproductive status, location of the lesion, preserved layering, presence/absence of a mass lesion, nature of the lesion and experience of the ultrasonographer) between technically successful and unsuccessful samplings. Odd ratios are presented with their 95% confidence intervals (95% CI). Continuous variables such as age, weight, body condition score and layer thickness were compared using the Mann–Whitney *U* test. Data are presented as frequencies and percentages for qualitative variables and median [range] for quantitative variables. Multivariable logistic regression was performed to assess for factors associated with technically successful GI wall FNA on selected variables based on previous literature, outcome of univariable analysis and authors' discussions (Turner et al., 2021). The model was further assessed for correlations and interactions. Statistical significance was defined as $p < 0.05$.

RESULTS

Interrogation of the cytology database, after removal of duplicates, returned 606 reports of individuals with potential cytology of the GI tract from percutaneous ultrasound-guided FNAs. Four hundred and fifty-six were excluded as not from the GI tract, three as impression smears and two due to incomplete data (cytology report unavailable and localisation of the lesion unavailable). A total of 70 cats and 75 dogs were included. Of these, two dogs and three cats had repeated sampling (same lesion twice); an additional cat was sampled on three occasions. The total number of cytology reports from the GI tract was 152.

Successful GI sampling

Eighty-eight GI submissions returned a cytology report and were considered successful (58%, 88/152). Of these, three diagnosed normal epithelium, 21 inflammation and 64 a neoplastic process (lymphoma $n = 42$, adenocarcinoma $n = 10$ and other neoplasia $n = 12$) (Table 1). Six cases had repeated sampling of the GI wall lesion, five were sampled twice, three were successful on second attempt, and one was sampled three times returning a cytology report on third attempt.

Locoregional lymph node FNAs and comparison with GI FNAs

Of the 145 individuals with GI wall FNAs, 84 had sampling of LLN. Sixty-seven LLN samples were successful (80%, 67/84); three diagnosed a normal node, 28 inflammation and 36 neoplasia (Table 1). Of 17 unsuccessful samplings, nine had successful GI wall FNAs. The remaining eight were unsuccessful for both GI wall and LLN FNAs.

Table 1. Summary of the cytological diagnosis obtained from gastrointestinal and lymph node fine needle aspirates (FNA)

	Gastrointestinal wall FNA		Lymph node FNA	
	n = 152	%	*n* = 84	%
Normal	3	2	3	4
Inflammatory	21	14	28	33
Neoplasia	64	42	36	43
Lymphoma	42		25	
Adenocarcinoma	10		5	
Other neoplasia	12		6	
Technically unsuccessful	64	42	17	20

Of the 67 successful LLN FNAs, 43 of 67 also had successful GI sampling. Both samples returned similar diagnosis in 30 of 43 cases (18 lymphomas, two adenocarcinomas, three other types of neoplasia and seven inflammatory processes). In 13 of 43 cases, samples from the GI tract and from the LLN, although deemed successful, did not yield identical results. Lymph node FNAs uncovered neoplasia in two cases diagnosed as inflammatory on GI FNA (one lymphoma and one mast cell tumour) and returned non-specific inflammation in nine cases with a diagnosis of GI neoplasia. The other two cases showed minor discrepancies (normal lymph node in face of GI inflammation and vice-versa).

The remaining 24 of 67 successful lymph node samples were from patients with unsuccessful GI wall FNAs. Thirteen returned non-specific inflammation and 11 neoplasia (seven lymphomas, two carcinomas and two other types of neoplasia).

Sampling of the LLN provided additional clinicopathological information in 52 of 145 cases (36%). In 30 cases, lymph node FNAs confirmed a pathological process (23 neoplasia and seven inflammatory processes), nine supported an absence of metastasis to the LLN, 11 diagnosed neoplasia where GI wall FNAs had been unsuccessful and two where the GI wall FNAs had returned inflammation.

Final diagnosis and diagnostic accuracy of gastrointestinal wall FNAs

Seventeen of the 88 cases with successful GI FNAs underwent subsequent histopathological characterisation of the lesion (endoscopic biopsies $n=6$, surgical biopsies $n=9$ or necropsy $n=3$) with one cat having both endoscopic biopsies and necropsy. Seven (7/17) showed complete agreement (one each of lymphoma, mast cell tumour, spindle cell tumour, adenocarcinoma and three inflammatory processes) and two (2/17) partial agreement; one cytological eosinophilic inflammation that was diagnosed as feline GI eosinophilic sclerosing fibroplasia (FGESF) and one malignant neoplasia suspected epithelial in origin on cytology which was mesenchymal on histopathology (hemangiosarcoma). Discrepancies were present in eight (8/17) cases. Seven diagnosed as inflammatory based on cytology were found neoplastic on histopathology (one lymphoma, four adenocarcinomas, one round cell tumour and one sarcoma). Endoscopic biopsies returned non-specific changes (ductal ectasia and mucosal fibrosis) in a case diagnosed with large cell lymphoma based on cytology. The strength of agreement between cytology and histopathology was poor, even when including the partial ones (Cohen's kappa coefficient of 0.08, 95% CI: 0.0 to 0.51 and 0.16, 95% CI: 0.0 to 0.58, respectively).

Thirty cases with technically unsuccessful GI wall FNAs had histopathology (endoscopic biopsies $n=17$, surgical biopsies $n=10$ or necropsy $n=3$). Eighteen diagnosed neoplasia (adenocarcinoma $n=6$, lymphoma $n=8$, other neoplastic diseases $n=4$) and 12 inflammatory conditions. Overall, final diagnosis was based on histopathology for 46 of 152 (30%), on cytology for 89 of 152 (59%) and was not reached in 18 of 152 (12%) of cases. The most common diagnosis was neoplasia 101 of 152 (66%) followed by nonspecific inflammation 32 of 152 (21%) (Table 2).

Predictors of successful gastrointestinal wall and lymph node FNAs

On univariable analysis, two ultrasonographic variables were significantly associated with successful sampling of the GI tract: the subjective description of a mass by the ultrasonographer in the imaging report (OR: 2.7, 95% CI: 1.4 to 5.3, $p=0.03$) and increased thickness of the layer ($p=0.0014$) (Tables 3 and 4). A higher number of submitted smears was associated with successful sampling, both for GI wall FNAs ($p=0.0027$) and for LLN ($p=0.042$). The nature of the lesion, based on final diagnosis, was not significantly associated with likelihood of success (Table 5).

Multivariable analysis (Tables 6 and 7) revealed significantly increased odds of technically successful GI wall FNAs with increasing number of smears taken (OR: 1.33, 95% CI: 1.06 to 1.68, $p=0.01$) and with the presence of a mass being described on imaging report (OR: 2.52, 95% CI: 1.14 to 5.59, $p=0.02$). Although GI wall thickness was significantly associated with success on univariable analysis, it was not significant on the final model. There was no interaction between GI wall thickness and subjective description of a mass on the imaging report ($p=0.36$) or number of smears ($p=0.95$), nor between number of smears and description of a mass ($p=0.16$). Due to the high a priori likelihood of interaction between GI wall thickness and subjective description of a mass, a model was constructed without subjective description of a mass. In this model, number of smears ($p=0.02$) and GI wall thickness ($p=0.03$) were statistically significant. To further interrogate the relationship between GI wall thickness and subjective description of a mass, a final model was constructed to look at predictors of a

Table 2. Breakdown of the final diagnosis and methods of obtention

	Histopathology	GI wall cytology	GI wall and LLN cytology	LLN cytology
Neoplasia	30	36	24	11
Adenocarcinoma	11	8	2	2
Lymphoma	10	21	20	7
Large cell	6	18	18	5
Large granular cell	0	2	2	2
Intermediate cell	2	1	0	0
Other types of lymphoma*	2	0	0	0
Other neoplasms	9	7	2	2
Inflammatory	16	6	4	6
Normal epithelium	0	1	0	0
Not reached	0	12	6	0
Total	46	55	28	17

*These two lymphomas were diagnosed as T-cell based on PARR (PCR for antigen receptor rearrangements)

Table 3. Observed categorical variables and influence on successful sampling

Observed variable	Category	Successful		Unsuccessful		Total	*p* Value	Odd ratio [95% CI]
		n	%	*n*	%	*n*		
Gastrointestinal wall								
Species	Dog	44	57	33	43	77	0.85	0.9 [0.5 to 1.8]
	Cat	44	59	31	31	75		
Sex	Male	49	56	39	44	88	0.52	0.8 [0.4 to 1.6]
	Female	39	61	25	39	64		
Reproductive status	Entire	12	55	10	45	22	0.73	0.9 [0.3 to 2.1]
	Neutered	76	76	54	42	130		
Location	Stomach	25	58	18	42	43	0.97	
	Duodenum	6	55	5	45	11		
	Jejunum	31	55	25	45	56		
	Ileocolic	11	61	7	39	18		
	Colon	15	63	9	37	24		
Layering	Preserved	28	50	28	50	56*	0.19	0.6 [0.3 to 1.2]
	Lost	55	61	35	39	90*		
Mass lesion	Subjectively described on imaging report	50	70	21	30	71	**0.03**	2.7 [1.4 to 5.3]
	Not described on imaging report	38	47	43	53	81		
	>15 mm cut-off†	33	66	17	34	50§	0.13	1.7 [0.9 to 3.5]
	<15 mm cut-off	53	53	47	47	100§		
	>20 mm cut-off‡	16	76	5	24	21§	0.06	2.7 [1.0 to 7.8]
	<20 mm cut-off	70	54	59	46	129§		
Experience of the ultrasonographer	Intern	14	52	13	48	27¶	0.59	
	Resident	58	58	42	42	100¶		
	Senior	14	67	7	33	21¶		
Locoregional lymph node								
Location	Gastric	3	50	3	50	6	0.31	
	Jejunal	36	82	8	18	44		
	Colonic	14	82	3	18	17		
	Other	14	82	3	18	17		
Echogenicity	Hypoechoic	38	84	7	16	45‖	0.2	2.0 [0.7 to 5.9]
	Not hypoechoic	27	73	10	27	37‖		
Homogeneity	Heterogenous	20	80	5	20	25‖	0.91	1.1 [0.3 to 3.4]
	Homogeneous	45	79	12	21	57‖		
Experience of the ultrasonographer	Intern	14	88	2	12	16	0.48	
	Resident	44	80	11	20	55		
	Senior	9	69	4	31	13		

*Preservation of the layering was not described and could not be assessed retrospectively in six cases
†Based on (Penninck et al., 2003)
‡Based on (Turner et al., 2021)
§Thickness of the gastrointestinal tract could not be retrieved in two cases
¶Identity of the imager was not outlined in four cases
‖Aspect of the lymph node on ultrasound was not available for two cases
Significant *p* values are in bold.

mass being described with species, ultrasonographer experience, GI wall thickness and description of loss of wall layering; only GI wall thickness was statistically significant ($p < 10^{-5}$). In no analysis was the site of FNAs significantly associated with success.

Development of complications

Twenty-four cases were euthanased within 48 hours of sampling based on poor prognosis (24/145, 17%). One-hundred and twenty survived to discharge (120/145, 83%). Complication was present in a single cat, euthanased within 12 hours of sampling after development of hypotension and haemoabdomen. However, FNAs of the liver had also been performed (diagnosed carcinoma with necrosis) thus, the bleeding cannot be strictly attributed to GI wall FNA.

In nine cases, complications could not be excluded. These were euthanased within 4 days of discharge with either unknown circumstance ($n = 2$ dogs), circumstances unlikely to be related to the GI FNA ($n = 5$, three dogs and two cats) and possible development of complication ($n = 2$). The latter were cats, one diagnosed with carcinoma that passed away within 12 hours of sampling (overnight, no post-mortem performed) and the second, diagnosed with large cell lymphoma in the liver, spleen and kidneys on concurrent FNAs that developed pyrexia and peritoneal effusion 4 days after sampling.

Absence of complication for the remaining 111 cases surviving hospital discharge was based on follow-up in 93/111, median of 34 days [4 to 1351]. The remaining 18 of 111 were either discharged on the same day ($n = 8$) or after overnight stay ($n = 10$) and deemed healthy. All cases were discharged with normal physical examination, on the day of sampling (61/111) or after overnight hospitalisation (50/111). Only nine individuals underwent point of care ultrasound.

Overall, 10 of 121 (8.3%) patients died or were euthanased after GI wall FNAs in circumstances where complications from sampling cannot be fully excluded. Cases were scrutinised by two authors (PH and GW) and three of 121 cases had complications possibly attributable to GI FNAs (2.5%; CI: 0.9 to 7.0).

Table 4. Observed continuous variables and influence on successful sampling

Observed variable	Successful			Unsuccessful			Total	*p* Value
	n	Median	Range	*n*	Median	Range	*n*	
Gastrointestinal wall								
Age (years)	88	10	3 to 17.5	64	10	0.25 to 16	152	0.51
Weight (kg)	86	5.2	2.2 to 36.8	64	5.8	2.4 to 42.2	150*	0.85
Dogs	42	11.9	2.2 to 36.8	33	17.6	3.5 to 42.2	75	0.37
Cats	44	3.8	2.7 to 7.5	31	3.7	2.4 to 5.9	75	0.35
Body Condition Score (/9)	79	4	1 to 8	63	3.5	1 to 7	142†	0.44
Thickness (mm)	86	12	2 to 50	64	9	2 to 52	150‡	**0.0014**
Number of smears	84	4	1 to 12	63	3	1 to 6	147§	**0.0027**
Locoregional lymph node								
Thickness (mm)	64	10	2.8 to 50	16	11.0	5.5 to 25	80¶	0.67
Number of smears	64	4	1 to 7	17	3	1 to 5	81‖	**0.042**

*Body weight was not recorded on two medical files
†Body condition score was not recorded on 10 medical files
‡Thickness of the gastrointestinal tract could not be retrieved in two cases
§Number of smears submitted was absent on five reports
¶Size of the locoregional lymph node could not be appraised in four cases
‖Number of smears submitted was absent on three records
Significant *p* values are in bold.

Table 5. Influence of the nature of the lesion on successful FNA sampling based on final diagnosis

Observed variable	Category	Successful		Unsuccessful		Total	*p* Value	Odd ratio [95% CI]
		n	%	*n*	%	*n*		
Gastrointestinal wall								
Nature of the lesion	Neoplastic	73	72	28	28	101*	0.096	2.1 [0.9 to 5.0]
	Inflammatory	15	56	12	44	27*		
Sub type of neoplasm	Lymphoma	44	76	14	24	58*	0.38	
	Adenocarcinoma	14	61	9	39	23*		
	Other types	15	75	5	25	20*		
Locoregional lymph node								
Nature of the lesion	Neoplastic	54	89	8	11	62†	0.16	2.9 [0.6 to 13.5]
	Inflammatory	7	73	3	27	10†		
Sub type of neoplasm	Lymphoma	38	86	6	14	44	0.95	
	Adenocarcinoma	9	90	1	10	10		
	Other types	7	88	1	12	8		

*Final diagnostic was not reached for 24 cases that had FNA of the gastrointestinal tract
†Final diagnostic was not reached in 12 cases that had FNA of the locoregional lymph node (six technically successful LN FNA that returned nonspecific inflammation with concurrent non diagnostic GI wall FNA and six unsuccessful samples)

Table 6. Multivariable logistic regression of potential predicting factors for technical success of FNA sampling of the GI wall

Variable	Odd ratio (OR)	Odd ratio 95% CI	*p* Value
Cat versus dog	0.88	0.42 to 1.85	0.74
Location versus stomach			
Duodenum	0.90	0.19 to 4.27	0.89
Jejunum	1.17	0.46 to 2.95	0.75
Ileum	2.04	0.56 to 7.42	0.28
Colon	1.11	0.35 to 3.51	0.26
Number of smears	1.33	1.06 to 1.68	**0.01**
Thickness (mm)	1.03	0.98 to 1.08	0.30
Subjective description of a mass on imaging report	2.52	1.14 to 5.59	**0.02**

Significant *p* values are in bold.

DISCUSSION

Percutaneous ultrasound-guided FNAs of GI wall lesions had a 58% success rate (88/152) in the present study; lower than reported by Turner et al. (30/44, 68%) and Crystal et al. (9/12, 75%). One explanation is the inclusion of a variety of GI lesions, including numerous minimally thickened layers (76/152 < 10 mm). Thicker lesions are associated with successful FNA sampling in the present study and previous veterinary literature and with successful ultrasound-guided core needle biopsies in human studies (Inoue et al., 2016; Turner et al., 2021). In human medicine, percutaneous methods are less commonly performed, therefore comparison is limited to a single case series reporting a diagnostic yield similar to the present study (26/44, 59%) (Carson et al., 1998).

Table 7. Multivariable logistic regression of potential predicting factors for technical success of FNA sampling of the GI wall excluding "subjective description of a mass on imaging report"

Variable	Odd ratio	Odd ratio 95% CI	*p* Value
Cat versus dog	0.81	0.39 to 1.66	0.56
Location versus stomach			
Duodenum	1.10	0.39 to 1.66	0.56
Jejunum	1.47	0.60 to 3.59	0.40
Ileum	2.41	0.69 to 8.41	0.17
Colon	1.39	0.46 to 4.25	0.56
Number of smears	1.30	1.03 to 1.63	**0.02**
Thickness (mm)	1.05	1.0 to 1.10	**0.03**

Significant *p* values are in bold.

The present study demonstrated significantly greater technical success when a GI mass was subjectively described on imaging report (50/71, 70%). This echoes the findings of Bonfanti et al., describing successful FNAs in 82% of cases (55/67 generated a cytological report) in confirmed GI neoplasms (Bonfanti et al., 2006). Although increased thickness of the GI wall was associated with increased technical success on univariable analysis, this was no longer significant on the initial multivariable model. Likewise, presence of a mass, using cut-offs of 15 or 20 mm, was not associated with improved technical success, in the literature or in the present work (Turner et al., 2021). In the absence of standardised definition, this suggests that the subjective description of a mass on ultrasound could better discriminate between neoplasia and benign lesions than lesion's thickness alone. However, this description is likely operator dependant; due to the high number of ultrasonographers in this study we were not able to assess at an individual level but ultrasonographer experience (lecturer vs. resident vs. intern) did not predict success. When subjective description of a mass was removed from the initial multivariable model, GI wall thickness and number of submitted smears remained significantly correlated with technical success, as previously described (Turner et al., 2021). Although the final diagnosis of neoplasia has previously been associated with more successful sampling, in the present study this was not the case (Table 5). This is unexpected as epithelial and round cell tumours are reputed to exfoliate better, resulting in more cellular preparations than mesenchymal ones (Baba & Câtoi, 2007). The last variable significantly associated with successful sampling was increased number of submitted slides which has previously been reported, both for ultrasound-guided sampling of GI lesions and for FNAs in general (Amores-Fuster et al., 2015; Turner et al., 2021). The veterinary literature does not offer recommendations on an ideal number of passages and subsequent number of submitted smears. Successful GI wall FNAs were obtained with a median of four slides (in the present work) to seven (Turner et al., 2021). However, more than the number, the quality of the samples (cellularity, preservation) defines technically successful FNAs. Therefore, in-house assessment of sample adequacy could be considered during the procedure to justify further attempts (Wypij, 2011).

Results of this study show that GI wall FNAs were less successful but have comparable accuracy to ultrasound-guided sampling of other organs. With diagnostic accuracy reported between 53% (9/17) in the present work and 67 (4/6) to 82% (9/11) previously, cytology of GI lesions is moderately to highly accurate (Crystal et al., 1993; Turner et al., 2021). In comparison, two of the most commonly aspirated abdominal organs are liver and spleen for which the reported success rate for ultrasound-guided FNA ranges from 61% (71/116) to 83% (183/220) and from 81% (76/94) to 90% (85/94), respectively (Cray et al., 2022; Llanos et al., 2023; Wypij, 2011). However, diagnostic accuracy of liver FNA is low, between 23% (49/220) in the most recent and largest study on the topic and 30% in an older publication (17/56) (Cray et al., 2022; Wang et al., 2004). The diagnostic accuracy of splenic cytology from ultrasound-guided samples has only been reported once, as 60% (21/35) (Watson et al., 2011).

Only a limited number of cases had both cytology and histopathology (30/64 unsuccessful GI wall FNA and 17/88 successful ones). Pursuing more invasive sampling is the next logical step after unsuccessful FNA but is not expected after successful ones unless the clinician has reasons to doubt the accuracy of cytology. Most of the cases undergoing biopsy sampling despite successful GI wall FNA were mass lesions described as inflammatory on cytology (11/17). The remaining were neoplasms needing further characterisation (6/17). Although the retrospective nature of this study precludes commenting on the clinical decision making, we hypothesise that biopsy sampling of the 11 presumed inflammatory lesions was motivated by concerns over the accuracy of GI wall FNAs and potential detrimental consequences of missing neoplasia on the patient's health. This scepticism is justified by our findings. A majority (7/8) of the incorrect diagnoses were inflammatory on cytology and found to be neoplastic on histopathology. This discrepancy could reflect inaccuracy of the FNA as well as non-representative sampling (periphery of the lesion, area of necrosis or inflammation). Orientation of the needle in several planes during sampling and acquisition of samples from various areas of the lesion is recommended to increase diagnostic yield and accuracy (Liffman & Courtman, 2017). A single case (1/8) was diagnosed with duodenal large cell lymphoma on cytology and inflammatory changes on histopathology from endoscopic biopsies (ductal ectasia and mucosal fibrosis). The cat underwent standard chemotherapy and diagnosis of lymphoma was confirmed on cytology at restaging. The risk of inaccurate diagnosis is a recognised limitation of endoscopic biopsies, which can result from sampling the wrong localisation or acquisition of inadequate biopsy samples (Evans et al., 2006).

Ultrasonographic appraisal of the LLN has been reported as beneficial in the investigation of feline chronic GI disease, rounded shape and a median size of 6.7 mm being suggestive of low-grade intestinal T-cell lymphoma (Freiche et al., 2021). However, the value of LLN sampling had not been previously assessed. In the present study, LLN sampling provided additional information in approximately a third of cases. The technical success of LLN FNAs was higher than GI wall FNAs, possibly as lymph nodes exfoliate high numbers of cells, but reactive changes and non-specific inflammation were commonly reported (Amores-Fuster et al., 2015; Cowell et al., 2003). Therefore, without histopathology a concordant

inflammatory cytology from both organs must be interpreted cautiously (risk of false negative). Similarly, neoplasia diagnosed on cytology from the LLN does not prove that the GI lesion is neoplastic nor that it is primary. Regardless of these limitations, LLN FNAs are regarded as technically simple, rapid, rewarding, associated with minimal complications and can provide a diagnosis in some cases where GI wall FNAs are not successful (Cowell et al., 2003). Thus, sampling of the LLN should be considered, particularly when enlarged and when GI wall FNAs appear acellular.

Assessment of complications was based on survival to discharge and additional retrospective review of medical files, the occurrence of minor or self-limiting complication was not specifically assessed and cannot be excluded. For 18 of 121 cases, follow-up was not available, and the absence of complication was postulated based on no owner contact or other report of concerns following discharge. This study revealed that assessment of post-FNA complications is not standardised in our institution. Only rare cases had repeated point of care ultrasound (9/111), but many patients spent at least one night hospitalised and had repeated clinical examination documenting the absence of pyrexia or haemodynamic instability (50/111). This limited assessment is supported by the current literature which suggests that complications following ultrasound-guided sampling of the GI tract are uncommon (none reported in three of the four published studies on the topic) and that when haemorrhage occurs, the lowest haematocrit is observed within 5 hours of sampling (Bigge et al., 2001; Bonfanti et al., 2004; Crystal et al., 1993; Turner et al., 2021). When considering abdominal ultrasound-guided biopsies, adverse events remain rare with major complication reported in 1.2% (3/233) and minor localised haemorrhage in 5.6% (13/233) (Léveillé et al., 1993). Due to the limitations of retrospective analysis, all cases where complications could not be fully excluded were recorded (8.3%, 10/121). We believe this may overestimate complication frequency as it includes seven cases where on review the clinical signs and subsequent euthanasia were considered unlikely to be related to sampling. The three remaining cases were a cat that developed life-threatening intra-abdominal haemorrhage possibly secondary to sampling of the liver rather than the GI tract and two additional cases that deteriorated (abdominal effusion and pyrexia) or died unexpectedly shortly after sampling for which complications remain plausible but progression of the primary disease cannot be excluded (neoplasia, low albumin, thrombus, etc). Thus, no cases had complications strictly attributable to GI wall FNAs.

Limitations of this study were mainly related to its retrospective nature. The searching strategy introduces a bias, as only samples submitted for cytology analysis can be included but not instances where GI wall FNAs were attempted, and slides were not submitted due to low cellularity. This may significantly artificially increase the observed GI wall FNA technical success and may also distort the complication rate observed for the procedure. Similarly, the terminology "technically successful FNA" was the object of discussions among the authors and with reviewers. Although imperfect, this denomination was chosen to best capture a FNA yielding enough cells to result in a cytology report. Acquisition and submission of a sample that returns a cytology report of "inadequate preservation or cellularity" is by definition "not technically successful."

The training status of the imager (intern, resident or board-eligible) did not correlate with increased technical success. An assessment of whether experience influenced diagnostic yield could have used number of years of experience rather than training status, but this was not available. Information on sampling method (with/without aspiration, size of needle and number of passes) was not available for review. Conflicting evidence has recently been published regarding the effect of needle gauge on cellularity and diagnostic yield and it is unclear whether aspiration, compared to no aspiration, results in superior quality samples (Bowlt Blacklock et al., 2018; Cray et al., 2022; Fleming et al., 2019; Launay et al., 2023; Llanos et al., 2023; Whitlock et al., 2021). Similarly, effect of the number of passes has not been evaluated in veterinary medicine, but in humans more than three passes was predictive for a correct diagnosis in endoscopy-guided sampling of lymph nodes and pancreatic lesions (van Riet et al., 2019). No consensus exists on the optimal needle gauge, technique (aspiration vs. no aspiration) and number of passes to sample the GI tract. Further studies are required to identify the influence of these factors and possibly optimise GI sampling.

Ultrasound-guided FNAs of GI wall lesions for cytology analysis had a lower technical success than FNA of other abdominal organs but comparable diagnostic accuracy. Sampling of the GI tract is likely associated with a low complication rate. As an affordable, widely available and relatively safe procedure, GI wall FNAs may have clinical use, particularly when confronted to a mass lesion or large GI thickening, especially when numerous samples can be acquired and a neoplastic aetiology is suspected. However, the pros and cons of undertaking GI wall FNA as an initial diagnostic test over biopsies should be carefully considered by the clinician and the limitations of the technique (risk of unsuccessful sampling or false negative if returning inflammation on cytology) should be clearly outlined to the owner. Sampling of the LLN is also safe and arguably easier than GI masses and, when included in patient investigation, provided additional clinicopathological information in a third of cases.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Author contributions

P. M. N. Henry: Conceptualization (equal); data curation (lead); investigation (lead); methodology (lead); project administration (lead); writing – original draft (lead). **A. M. Boag:** Formal analysis (equal); supervision (supporting); validation (supporting); writing – original draft (supporting); writing – review and editing (supporting). **J. R. S. Dandrieux:** Validation (supporting); writing – review and editing (supporting). **R. Rossi:** Data

curation (supporting); investigation (supporting). **G. A. Woods:** Conceptualization (equal); supervision (equal); writing – review and editing (equal).

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [PH].

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