

# Peculiar Histopathological Alterations of Enterocytes in A Coronavirus Disease 2019 Patient with Mycobacterial Tuberculosis Co-Infection

Rana AL-Zaidi (✉ [angelysurgery@yahoo.com](mailto:angelysurgery@yahoo.com))

King Faisal Hospital

Nasir AL-Noor

King Faisal Hospital

Adel Habbash

King Faisal Hospital

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## Research

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# Abstract

## Background:

The ongoing novel Coronavirus Disease 2019 (COVID-19) pandemic is principally defined by its respiratory symptoms. While it is clear that the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) can affect the gastrointestinal tract (GIT) and the pathogenesis of coronavirus disease 2019 is better understood, the exact pathological alterations following infection require further investigation. The purpose of this paper is to report and share our histopathological findings from a right hemicolectomy specimen of a confirmed COVID-19 positive case, which exhibited a *Mycobacterium Tuberculosis* co-infection.

## Methods:

Microscopic sections from right hemicolectomy specimen were appropriately stained and studied by two anatomical pathologists. Additionally, we searched PubMed and Google Scholar databases for reports/observations regarding pathological alterations of the intestine following COVID-19 infection.

## Results:

Histological sections showed novel peculiar pathological alterations in the terminal ileal mucosa involving principally absorptive enterocytes with evidence of striking cellular injury as well as prominent erythrophagocytosis in the mesenteric lymph nodes. No specific pathological alterations were observed in the appendix or colon. The characteristic pathological features of *Mycobacterium Tuberculosis* infection were also observed throughout the specimen.

## Conclusions:

Our observations showed that the novel SARS-CoV-2 can affect the gastrointestinal tract, causing epithelial injury and pathological alterations attributed to its ability to infect absorptive enterocytes by interacting with the Angiotensin Converting Enzyme-2 (ACE2) receptor. These pathological findings could be regarded as viral cytopathic changes and should be considered when evaluating gastrointestinal specimens from COVID-19 infected patients.

## Introduction

The ongoing global pandemic of COVID-19, caused by the novel SARS-CoV-2, has rapidly spread worldwide. It has infected more than 100 million individuals, leading to more than 2 million deaths. SARS-CoV-2 is associated with the development of a systemic disease that typically affects the respiratory tract. The most common symptoms are fever, dry cough, fatigue, headache, and myalgia. The gastrointestinal tract has a lower incidence, either as a concurrent infection with the respiratory tract or as the initial presentation of COVID-19 infection. The range of GIT symptoms included diarrhea, abdominal pain, nausea, vomiting, and anorexia [1]. While the virus is highly contagious and spreads predominantly

by respiratory droplets and aerosols, SARS-CoV-2 has been isolated from stool samples, suggesting that the virus could be transmitted via the fecal-oral route [2]. Continuous investigation of the pathogenesis of GIT infections caused by SARS-CoV-2 led to a better understanding. However, the histopathological alterations of the gastrointestinal mucosa have not been well elucidated. We observed peculiar pathological alterations of the terminal ileal mucosa and the mesenteric lymph nodes, which were part of a right hemicolectomy specimen performed for bowel perforation secondary to GIT infection with *Mycobacterium Tuberculosis* in a COVID-19 positive patient. Our aim was to share these microscopic findings with practicing pathologists and improve the awareness of what they can expect to see in gastrointestinal biopsies and resections of COVID-19 infected patients.

## Material And Methods

A right hemicolectomy specimen was fixed in 10% neutral buffered formalin, appropriately examined, and sectioned. Representative sections were then processed, microtome cut at a thickness of four-micrometer, and hematoxylin-eosin stained. Four-micrometer sections on charged slides were evaluated by immunohistochemistry for CD10 (clone 56C6, Dako, prediluted), Ep-CAM (clone Ber-EP4, Ventana, prediluted), EMA (clone GP1.4, Leica, prediluted), E-cadherin (clone 36, Ventana, prediluted), and Ki-67 (clone MM1, Leica, diluted 1:50). Sections on regular slides were stained with periodic acid-Schiff histochemical stain and Ziehl-Neelsen stain.

## Results

### Pathological Findings

Microscopic examination of the terminal ileum, cecum, ascending colon, and appendix revealed multiple caseating epithelioid granulomas scattered throughout the wall. The overlying mucosa was ulcerated, with granulation tissue formation. Numerous acid-fast bacilli within the epithelioid granulomas were observed following Ziehl-Neelsen staining. The remainder of the terminal ileum mucosa showed patchy peculiar architectural alterations with normal intervening mucosa (Fig. 1). The villi of the affected mucosa showed shortening, widening, and blunting (Fig. 2). There was a striking "hobnail" appearance with focal detachment/denudation of enterocytes, erosions, and fibrinopurulent exudate (Fig. 3). Loss of microvilli and goblet cells were also observed (Fig. 4). The underlying lamina propria was expanded by marked edema and congestion with occasional thrombosis of the blood vessels (Fig. 5). Crypt regeneration and elongation was observed. The high-power view showed striking cytological alterations of the absorptive enterocytes including cuboidal to rounded appearance with striking nuclear enlargement, coarse chromatin pattern, prominent nucleoli, and thickening of the nuclear membrane (Fig. 6). A subset of absorptive cells showed binucleation. Predominantly subnuclear intracytoplasmic vacuoles with a signet ring-like appearance were apparent (Fig. 7). Periodic acid-Schiff staining showed positive intracytoplasmic material in scattered enterocytes, which might represent viral glycoproteins (Fig. 8). Sections from the mesenteric lymph nodes showed involvement by caseating granulomatous

inflammation. In addition, the nodal trabecular and medullary sinuses were expanded with histocytes that showed prominent erythrophagocytosis (Fig. 9).

## Immunohistochemical Evaluation

We observed a diminished to complete loss of the normal linear brush-border staining pattern of CD10, in contrast to the adjacent normal mucosa (Fig. 10). Furthermore, diminished to complete loss of the normal basolateral immunohistochemical staining pattern of Ep-CAM and the normal diffuse pattern of E-cadherin staining was noted in the infected cells (Figs. 11 and 12, respectively). We observed aberrant cytoplasmic expression of EMA in a subset of infected cells, in contrast to the limited staining of the luminal surface of the normal crypts (Fig. 13). The Ki-67 labeling index was noted to extend upward toward the surface of the villi, in contrast to the limited staining pattern in the base of normal crypts (Fig. 14).

## Discussion

The novel SARS-CoV-2 causes the COVID-19, which has rapidly spread worldwide, threatening global health. SARS-CoV-2 is a single-stranded, enveloped RNA virus that belongs to the betacoronavirus 2b lineage [1, 3]. Its diameter is approximately 65–125 nm. Structurally, it has four main proteins, including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein. The S glycoprotein is a transmembrane protein that protrudes from the viral surface and facilitates binding and entry of the virus into host cells by interacting with the ACE2 receptor [4]. According to the Human Protein Atlas database ([proteatlas.org](http://proteatlas.org)), ACE2 is widely expressed in various human organs and tissues, including the oral and nasal mucosa, nasopharynx, lung, small intestine, colon, liver, spleen, kidney, and brain. It has been shown that the ACE2 expression is approximately 100-fold higher in the GIT than in the respiratory system. This makes GIT highly susceptible to SARS-CoV-2 infections. ACE2 is expressed on the luminal surface of the absorptive enterocytes of the small intestine and colon, with lower expression in crypt epithelial cells [5]. A previous study showed that both RNA and protein expression of ACE2 is higher in the small intestine than that in the colon [1]. ACE2 plays an important role in the regulation of dietary amino acid homeostasis, innate immunity, microbial ecology, and susceptibility to colitis [5].

SARS-CoV-2 has a tropism to the GIT, similar other members of the coronavirus family, although it affects the GIT at a lower frequency than the respiratory system. Up to 30% of patients with pulmonary infection complain of GIT symptoms, mostly in association with respiratory symptoms; however, only 4% of patients complain of GIT symptoms alone [6]. The most common GIT symptoms were nausea and vomiting (41.6%), diarrhea (17.2%), abdominal pain, and anorexia [1]. Several studies have documented the presence of SARS-CoV-2 RNA in stool or anal/rectal swabs in COVID-19 patients and suggested that the virus can replicate and exist in the GIT [7].

The pathogenesis associated with the GIT infection with SARS-CoV-2 mainly relies on the entry of the virus into the cytoplasm of absorptive enterocytes through its interaction with ACE2. Successful virus entry depends not only on the ACE2 receptor, but also on endogenous serine proteases such as furin and cellular transmembrane protease serine 2 (TMPRSS2), which cleaves the S protein of SARS-CoV-2 into two segments (S1 and S2) [4, 7]. Both furin and TMPRSS2 are widely distributed in the small bowel mucosa. This cleavage is critical for the attachment of the virus to both the ACE2 receptor and the cellular membrane. This attachment is followed by endocytosis of viral genomic material (RNA). Next, the viral mRNA is translated into new structural proteins with subsequent insulation in the endoplasmic reticulum-Golgi intermediate compartment, from which they form small vesicles that eventually undergo exocytosis [4, 7]. This explains the presence of intracytoplasmic vesicles/vacuoles that are noted in the affected enterocytes from our samples, some of which contain periodic acid-Schiff positive material that represent the newly formed structural glycoproteins of SARS-CoV-2. Furthermore, the partial or complete blockage of the ACE2 receptor by a large viral load impairs the host cell nourishment supply and capabilities necessary to ensure a balanced immune response. The transport of amino acids, particularly tryptophan, is also impaired. This results in aberrant mTOR activation and impaired expression of antimicrobial peptides from Paneth cells, which eventually leads to alterations in the gut microbial environment [5, 7]. The infected cells probably undergo subcellular alterations that might lead to certain pathological changes, as seen in our case. One of these alterations represents the loss of brush borders of the enterocytes, which is known to occur in the setting of active enteritis and is the leading cause of diarrhea. This alteration was best evaluated by performing CD10 immunohistochemical staining. CD10 is a membrane-associated neutral peptidase which is normally observed as a linear brush-border staining pattern of the small intestinal mucosa. Variable loss of brush-border immunostaining for CD10 is usually observed in the setting of active enteritis [8]. Another alteration is represented by the reduced expression of E-cadherin. E-cadherins are a major constituent of adherens junctions and play an essential role in intestinal homeostasis by providing mechanical integrity as well as maintaining cell polarization. Additionally, E-cadherins are necessary for the proper maturation and differentiation of secretory cell lineages, including Paneth and goblet cells. Downregulation of E-cadherin function due to decreased expression occurs secondary to the effect of inflammatory cytokines that lead to the activation of signaling pathway mediators, such as GTPases, which in turn destabilize adherens junctions and disrupt cell contacts; thus, facilitating pathogen invasion. Loss of E-cadherin function has been reported in inflammatory conditions of the intestine, such as Crohn's disease and ulcerative colitis [9]. This explains the obvious detachment and "hobnailing" appearance of the enterocytes as well as the diminished immunostaining for E-cadherin seen in our case. Similarly, possible alterations in the expression of EpCAM, which is a transmembrane glycoprotein, may occur secondary to SARS-CoV-2 infection. EpCAM plays an important role in intercellular adhesion, cell signaling, proliferation, differentiation, and cell polarity. Furthermore, EpCAM is enriched in the basolateral membrane of the intestinal absorptive cells [10]. Diminished or loss of immunostaining for this adhesion molecule was noted in our case. This suggests that significant subcellular alterations are induced by SARS-CoV-2 infection. In addition, *Mycobacterium Tuberculosis* co-infection certainly played a significant synergistic role in causing the overwhelming SARS-CoV-2 infection in this case.

## Review of Literature

Few studies describing the pathological findings of SARS-CoV-2 in the intestine have been reported to date. Liu et al. described an autopsy finding from a COVID-19 patient who developed alternating segmental dilation and stenosis of the small intestine [11]. Xiao et al. performed an endoscopy of the GIT for a confirmed COVID-19 patient. They observed damage to the esophageal mucosa, numerous plasma cells, as well as lymphocytes in the lamina propria of the stomach, duodenum, and rectum. Furthermore, viral nucleocapsid proteins were detected in the cytoplasm of the infected cells [12]. A reported case of acute hemorrhagic colitis showed a slight expansion of the lamina propria due to edema with normal cellularity and intact crypts was found [13]. A study of 67 autopsies performed on patients who died due to COVID-19 infection revealed no gross or microscopic abnormalities of 16 small intestine and 17 colons examined. However, hemophagocytic histiocytes (with engulfment of red blood cells) were noted in the spleen (9 out of 22 cases), bone marrow (4 out of 6 cases), thoracic lymph nodes (9 out of 11 cases), liver Kupffer cells, and epicardial inflammation in one case. According to this observation, the study suggested that SARS-CoV-2 may induce a macrophage activation syndrome-like state, which might occur secondary to the cytokine storm [14].

## Conclusion

COVID-19 is a threatening disease that causes systemic illness. It primarily affects the respiratory system; however, the gastrointestinal tract is another affected site. While the pathogenesis of this disease becomes more evident, it appears that the pathological alterations of the small intestinal epithelium and the mesenteric lymph nodes are associated with the direct injury induced by the virus on the host cells, as well as the effect of the aberrant inflammatory response produced by the host's immune system. We suggest that SARS-CoV-2 might cause cellular and subcellular alterations that induce certain microscopic changes in the affected tissue. The pathological alterations of the intestine associated with the SARS-CoV-2 infection are important to be known by practicing anatomic pathologists; thus, they deserve close attention and further investigation.

## Abbreviations

**COVID-19:** coronavirus disease 2019

**SARS-CoV-2:** severe acute respiratory syndrome coronavirus 2

**GIT:** gastrointestinal tract

**ACE2:** Angiotensin Converting Enzyme-2 receptor

**TMPRSS2:** transmembrane protease serine 2

## Declarations

- **Ethics approval and consent to participate:** Not applicable.
- **Consent for publication:** written informed consent for publication was obtained.
- **Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
- **Funding:** Nil
- **Authors' contributions:** RZ performed the histopathological examination of the specimen and noticed the presence of the peculiar pathological alterations described in the manuscript and performed the appropriate stains. RZ is a major contributor in writing and preparing the manuscript. NN was a contributor in evaluating the histological sections and concurred the presence of such novel findings. NN contributed in the literature search as well as in writing the manuscript. AH was contributed in the literature search and in revising the manuscript. All authors read and approved the final manuscript.
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## Figures

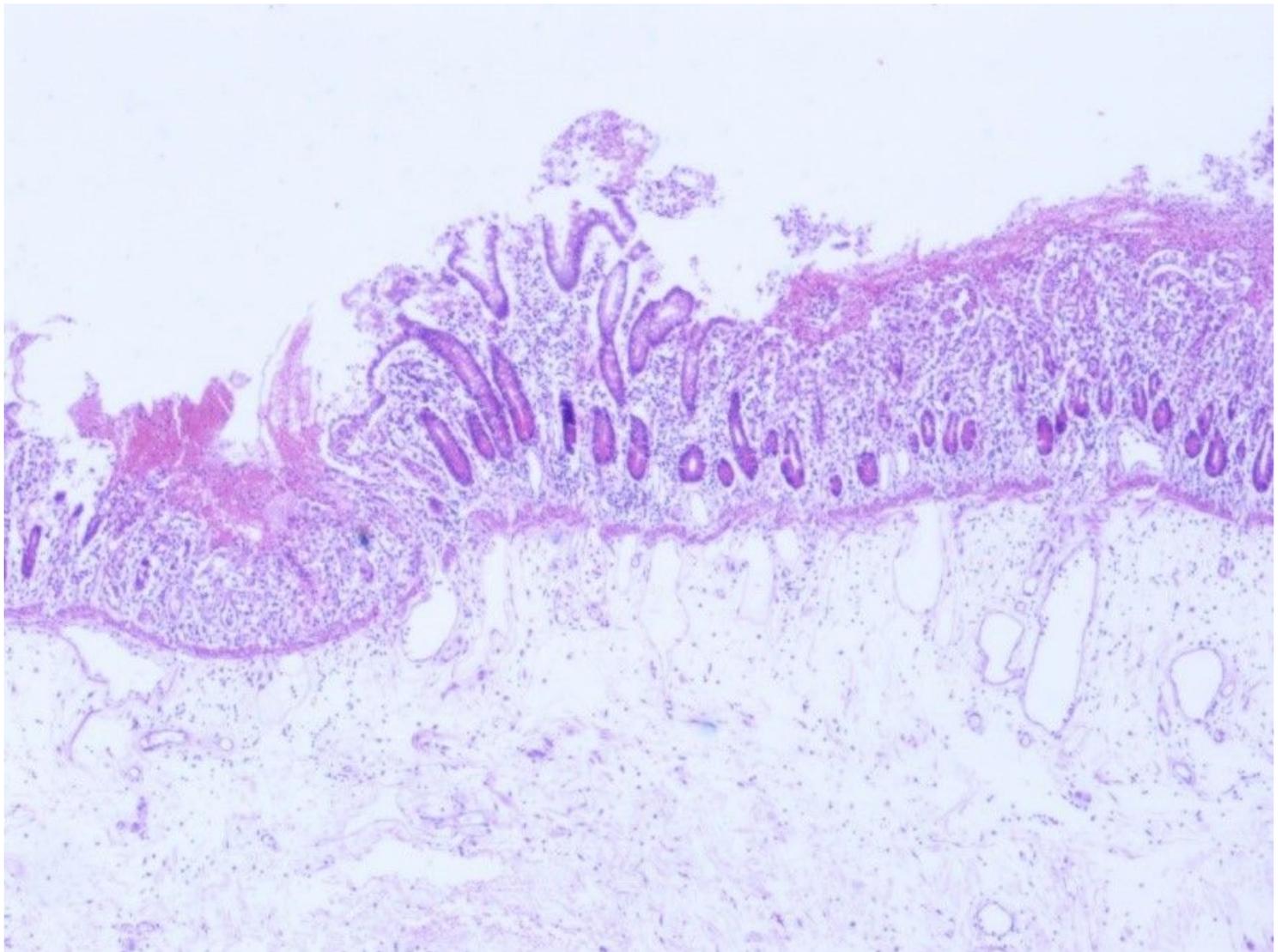
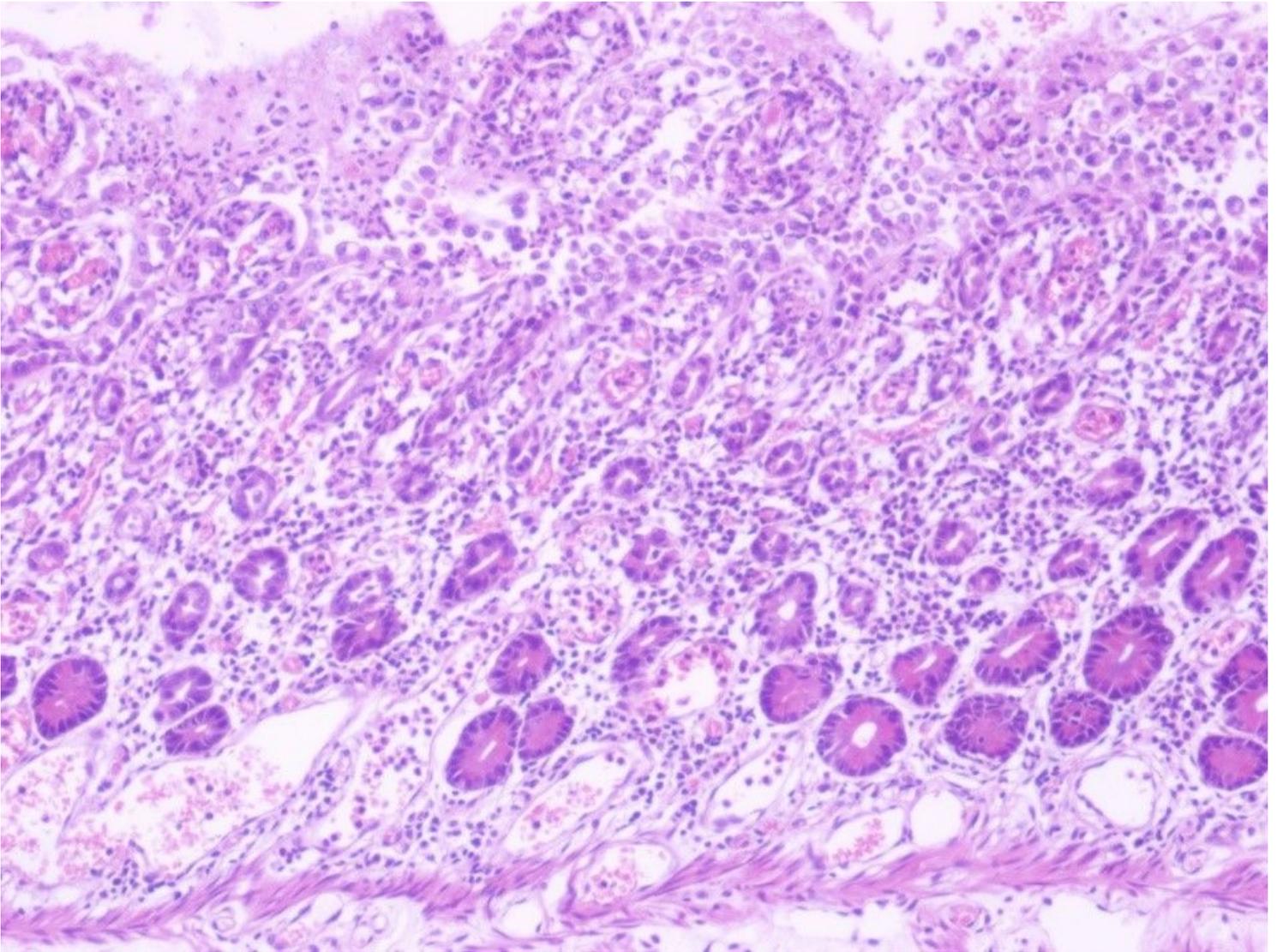


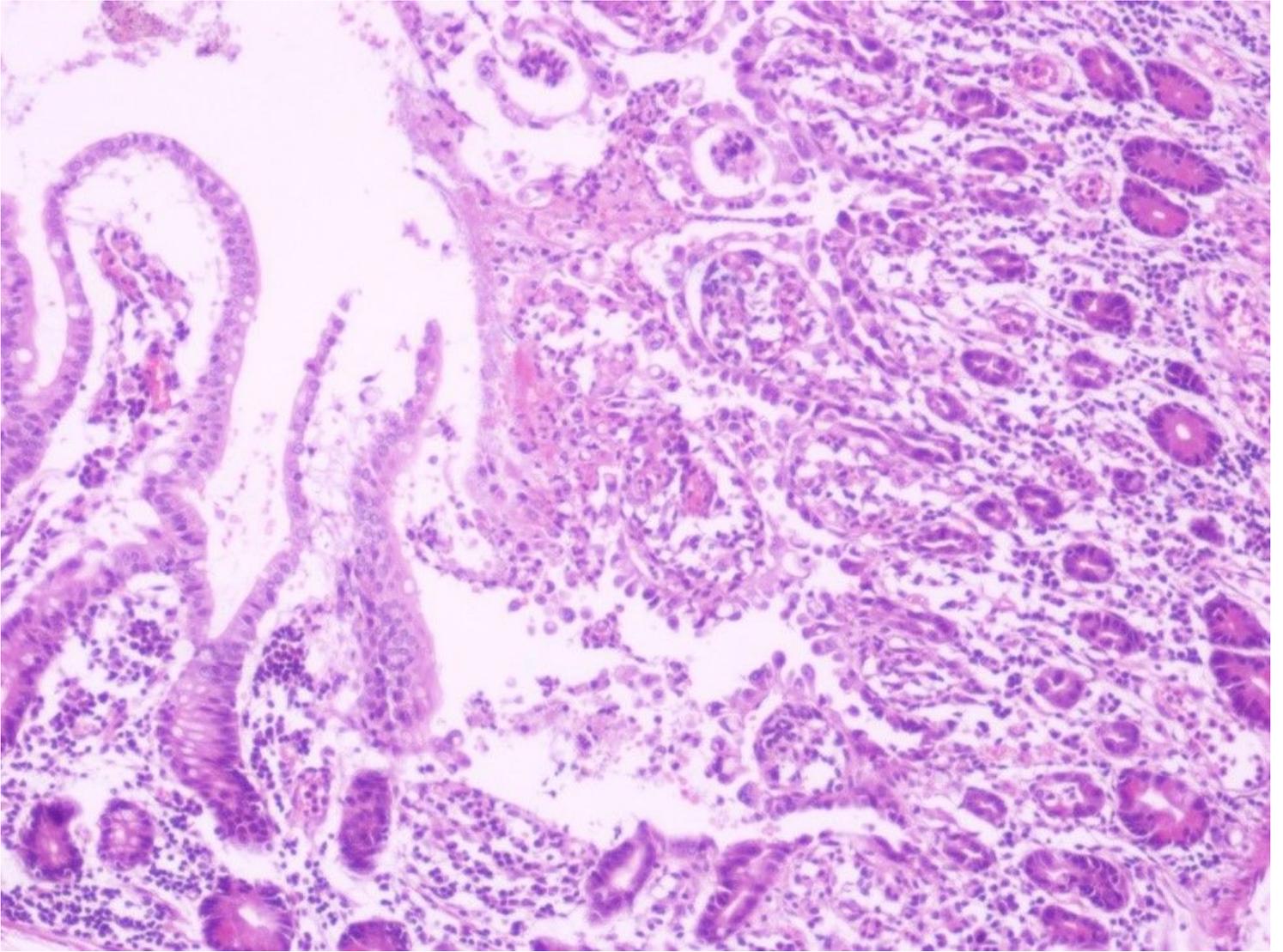
Figure 1

Section from the terminal ileum shows mucosal erosion with overlying fibrino-purulent exudate, loss of villous architecture, and crypts elongation. An intervening un-involved mucosa at the center of the image is noted (hematoxylin-eosin, original magnification x40).



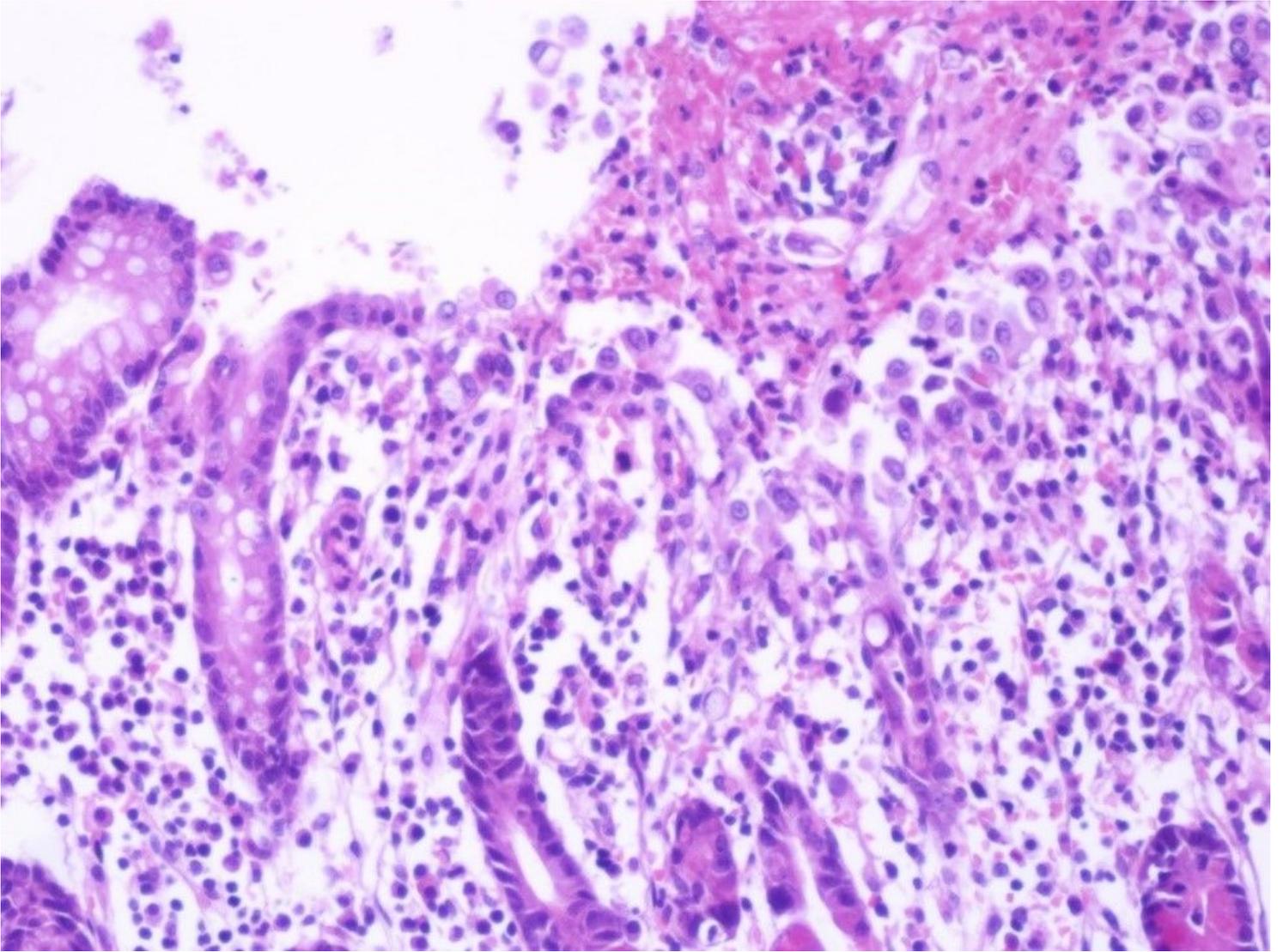
**Figure 2**

Marked villous blunting, denuded surface epithelium, expansion of lamina propria by edema, chronic inflammatory cells, and vascular congestion (hematoxylin-eosin, original magnification x100).



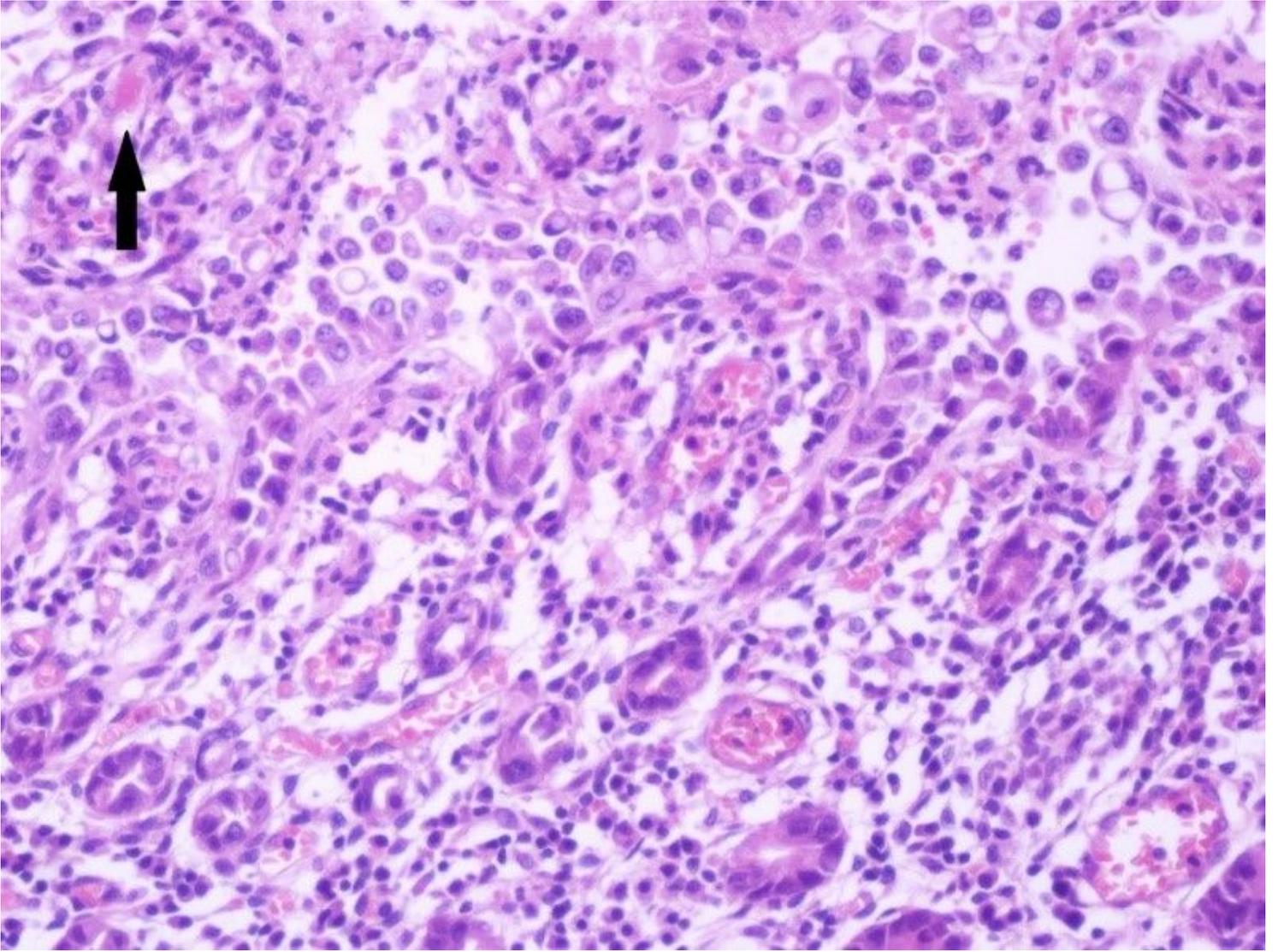
**Figure 3**

Villous blunting, erosion with fibrino-purulent exudate, lamina propria edema, and regenerated crypts, compared to normal mucosa at left side of image (hematoxylin-eosin, original magnification x100).



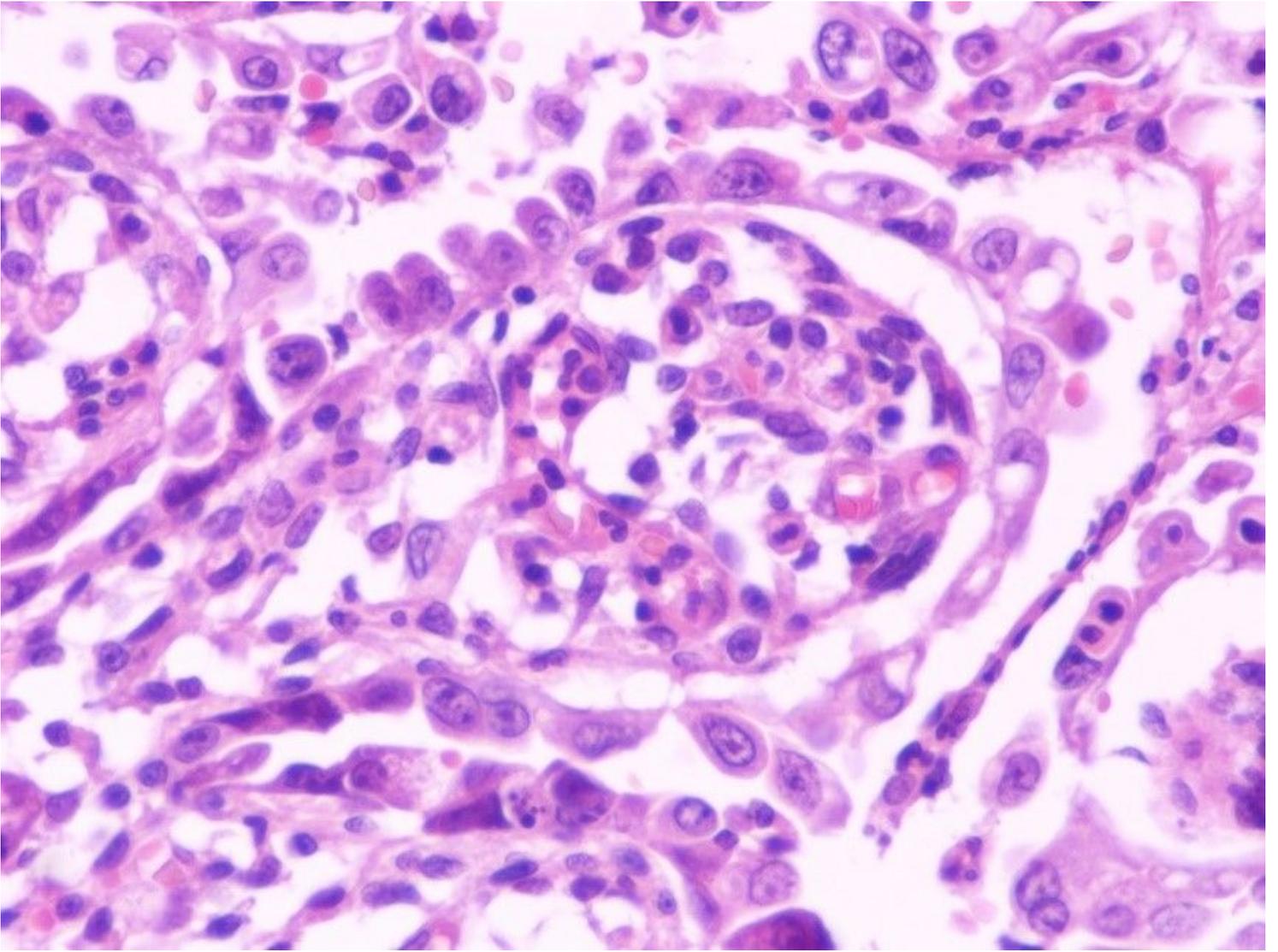
**Figure 4**

Villous blunting, erosion with fibrino-purulent exudate, lamina propria edema, and regenerated crypts, compared to normal mucosa at left side of image (hematoxylin-eosin, original magnification x100).



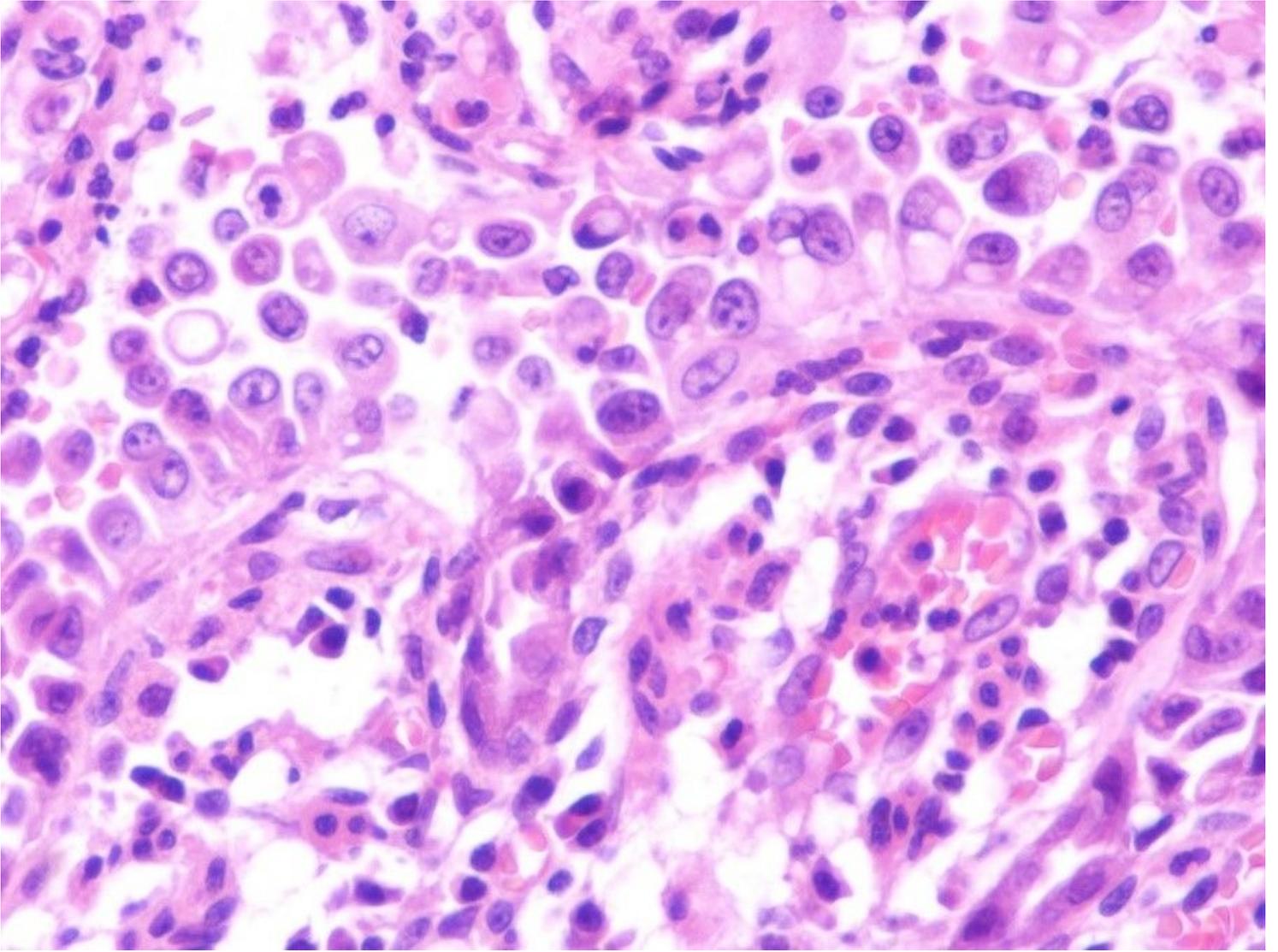
**Figure 5**

Cuboidal enterocytes with denudation/detachment, blunting and widening of villi by edema, chronic inflammation, and vascular congestion. Occasional vascular thrombi (arrow) (hematoxylin-eosin, original magnification x200).



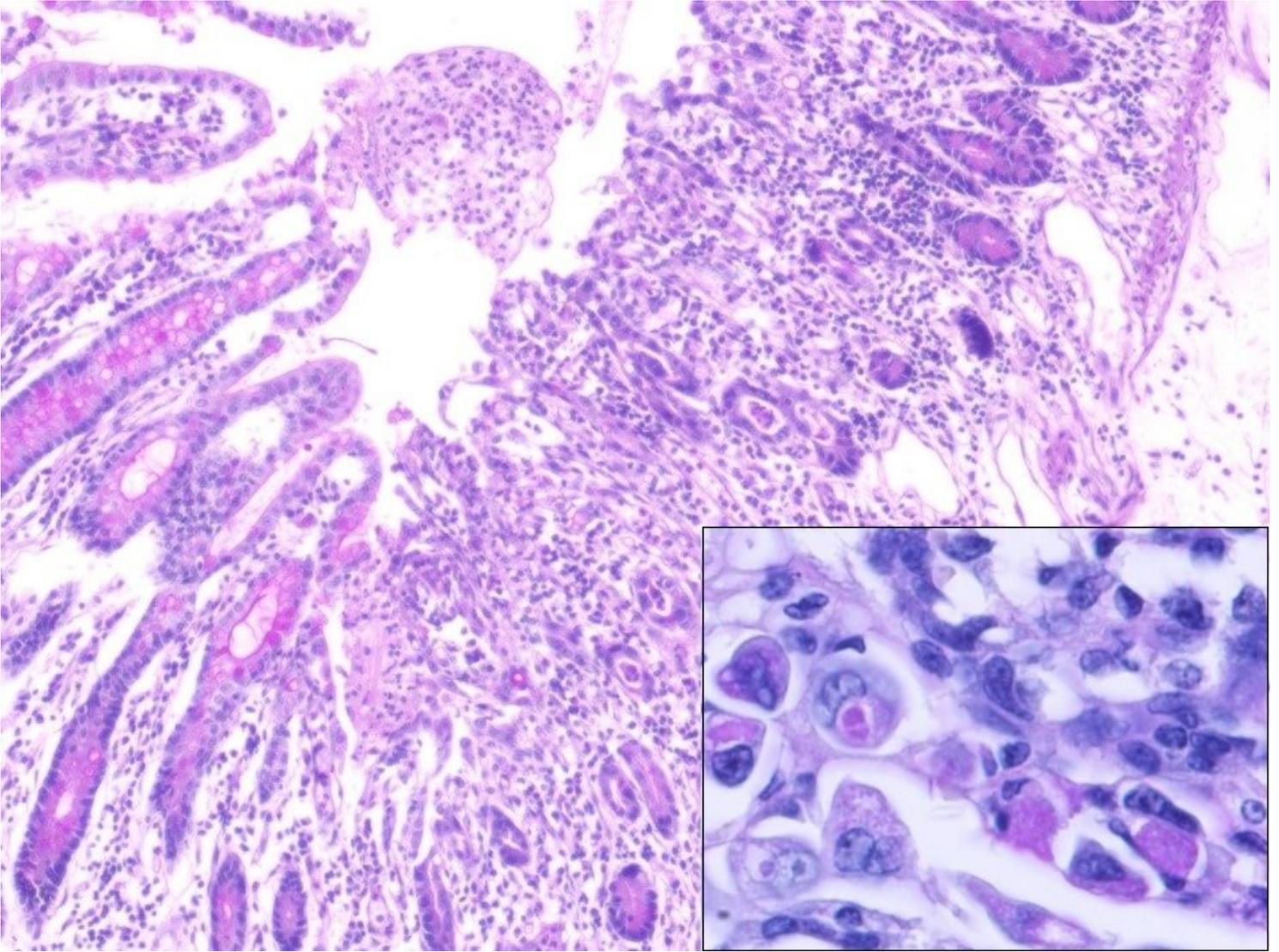
**Figure 6**

Infected enterocytes exhibit cuboidal to rounded appearance, loss of microvilli, cytoplasmic vacuoles, reactive-type nuclear atypia, and loss of polarity (hematoxylin-eosin, original magnification x400).



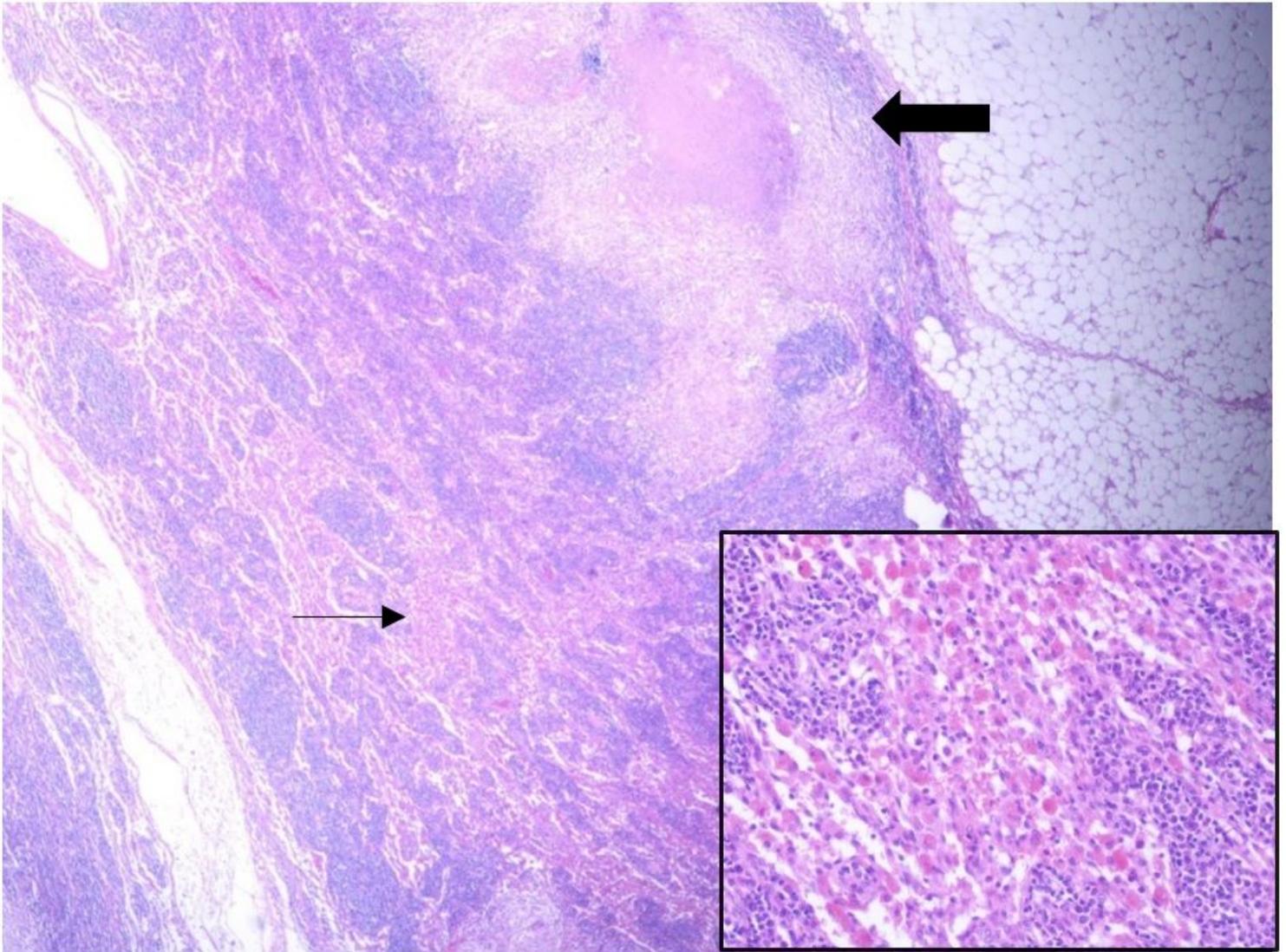
**Figure 7**

Infected enterocytes exhibit cuboidal to rounded appearance, loss of microvilli, cytoplasmic vacuoles, reactive-type nuclear atypia, and loss of polarity (hematoxylin-eosin, original magnification x400).



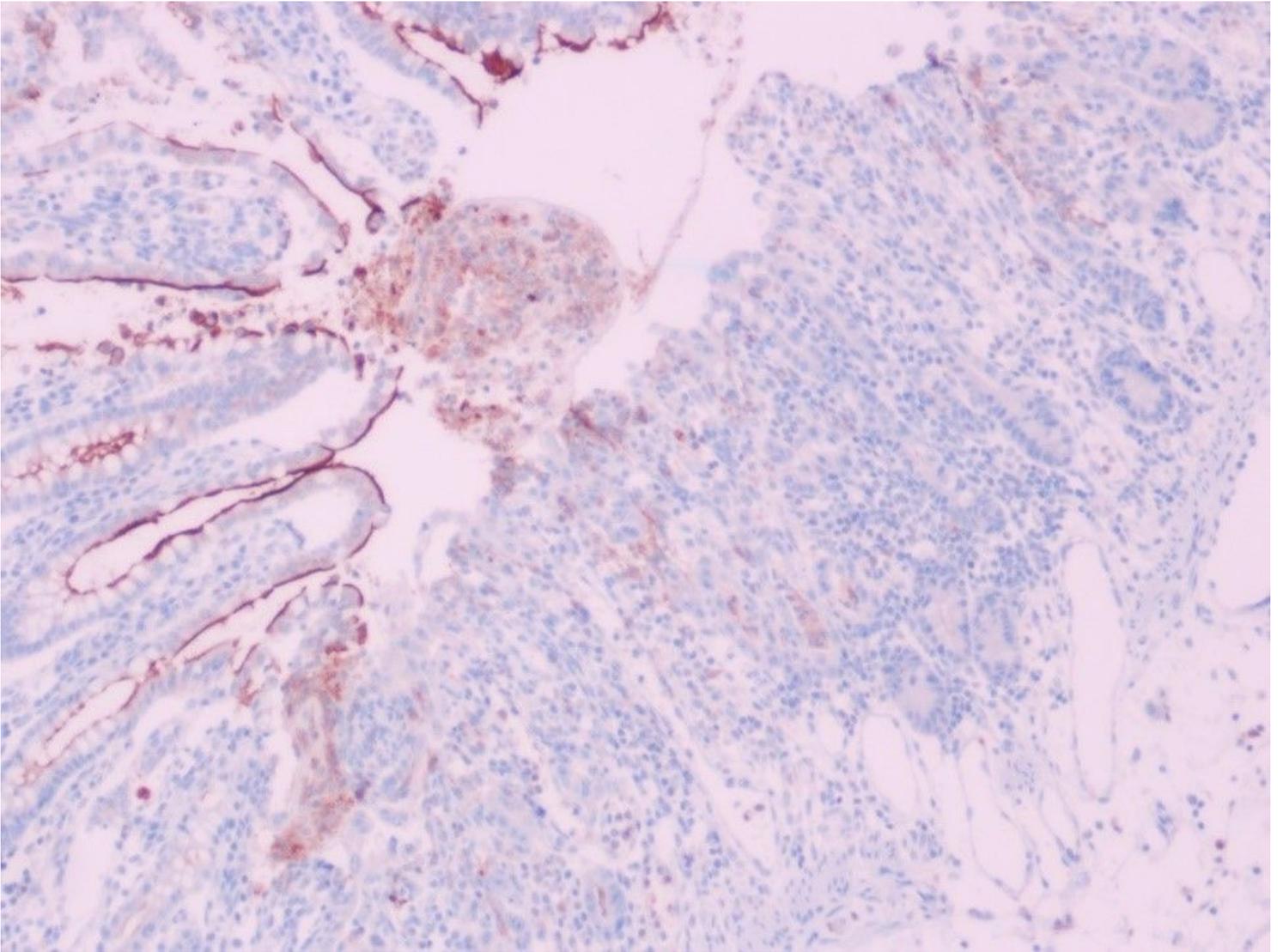
**Figure 8**

Loss of cytoplasmic mucin of enterocytes with diminished goblet cells as compared to normal mucosa at left side of image. Intracytoplasmic material that could represent SARS-CoV-2 glycoprotein (inset) (Periodic acid-Schiff stain, original magnification x100 and x400 [inset]).



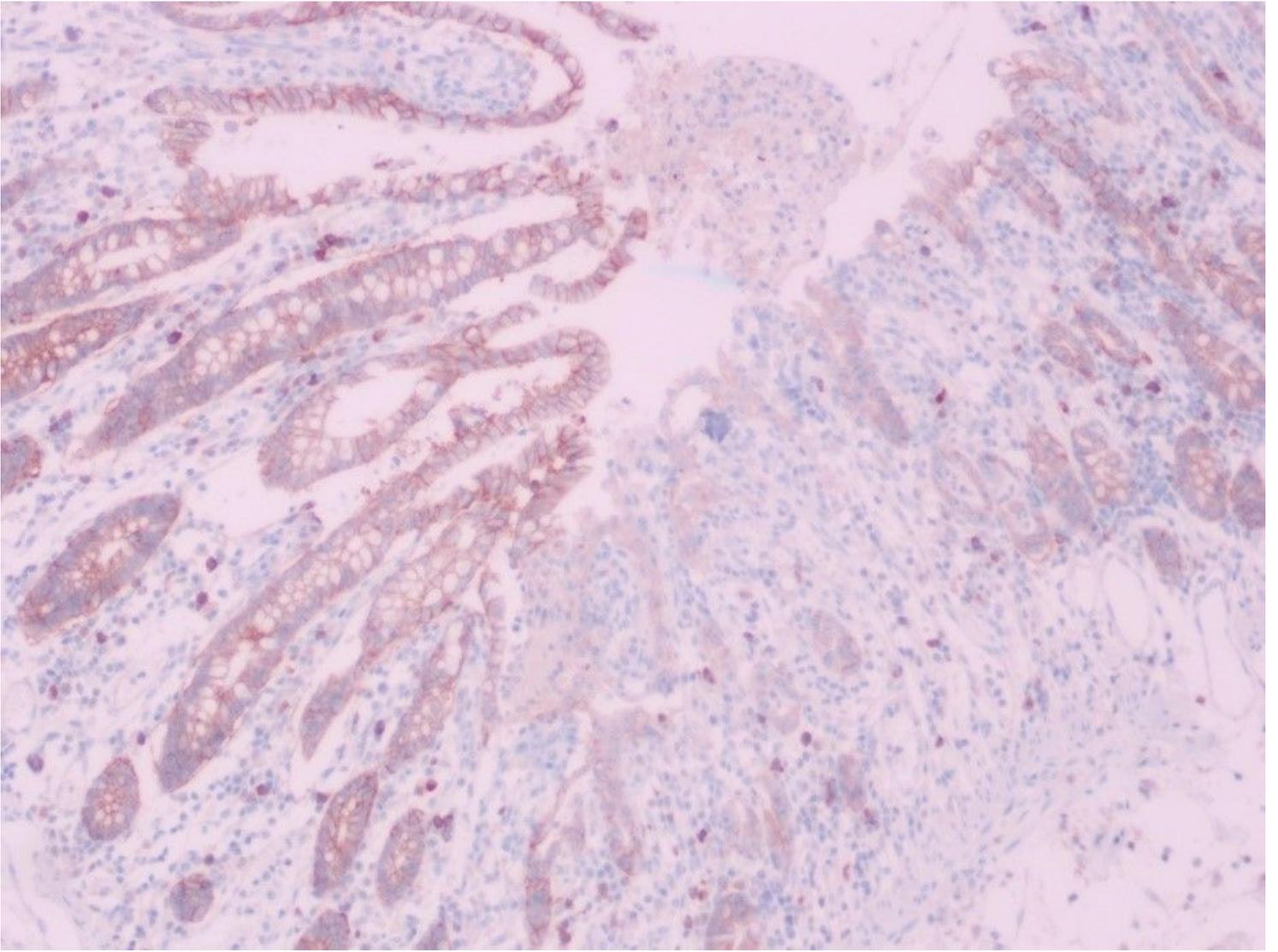
**Figure 9**

Mesenteric lymph node with a necrotizing granuloma (thick arrow) and expansion of sinuses by histiocytes (thin arrow). Erythrophagocytosis expanding the nodal sinuses (inset) (hematoxylin-eosin, original magnification x20 and x200 [inset]).



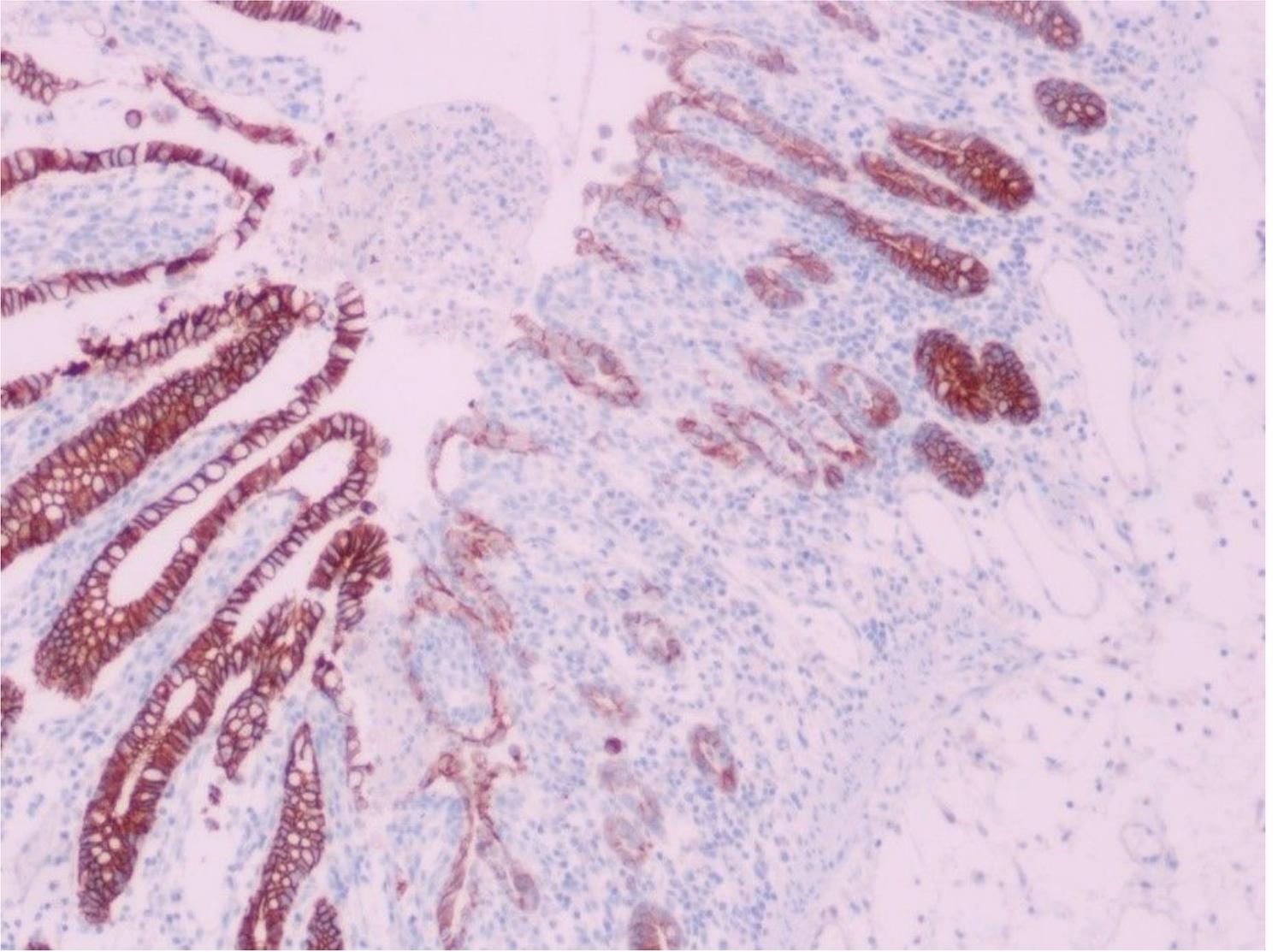
**Figure 10**

Complete loss of the normal linear brush-border staining pattern of CD10 in the infected enterocytes, compared to the normal mucosa at left side of image (original magnification x100).



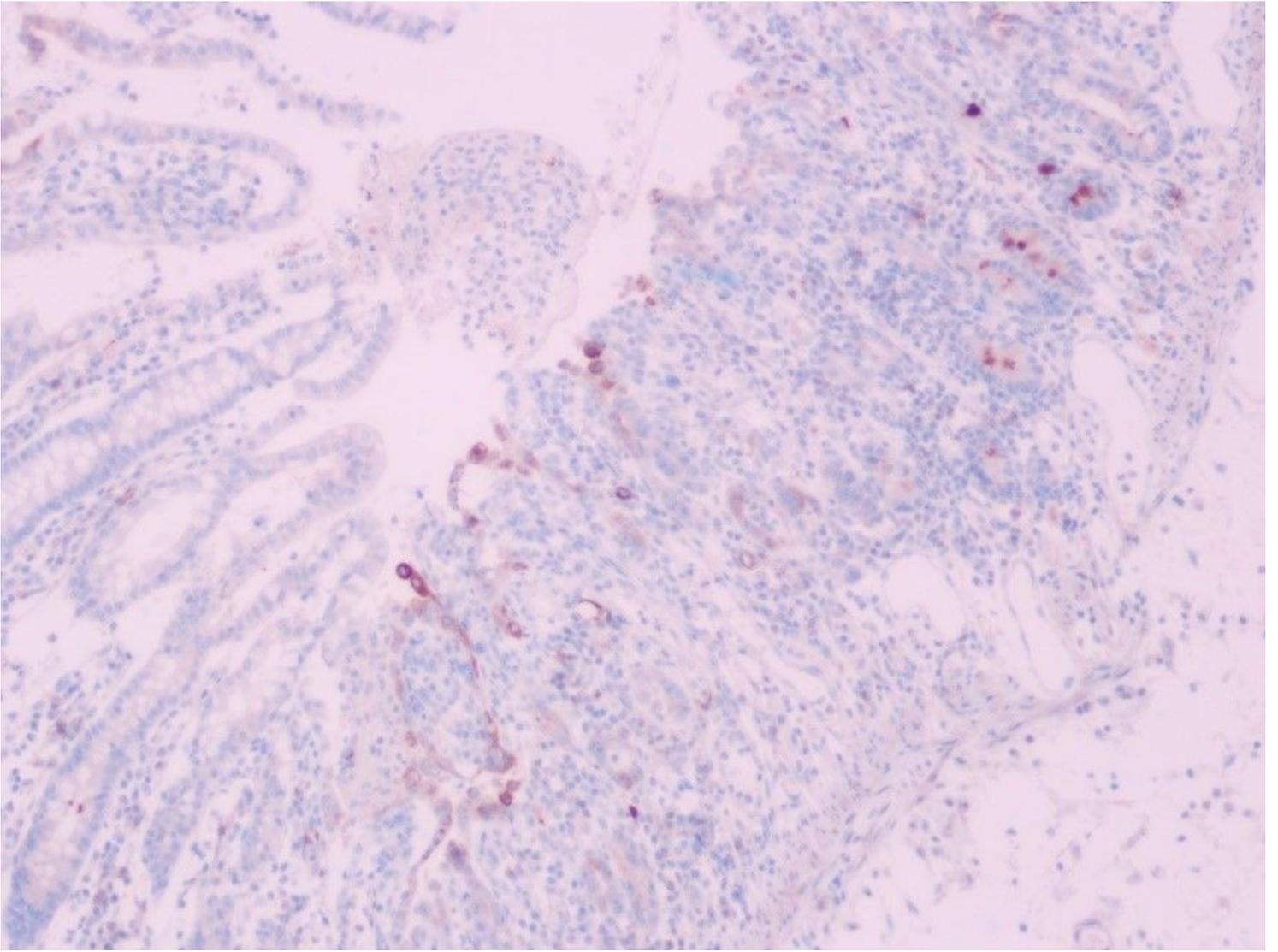
**Figure 11**

Loss of the normal basolateral immunohistochemical staining of Ep-CAM (original magnification x100).



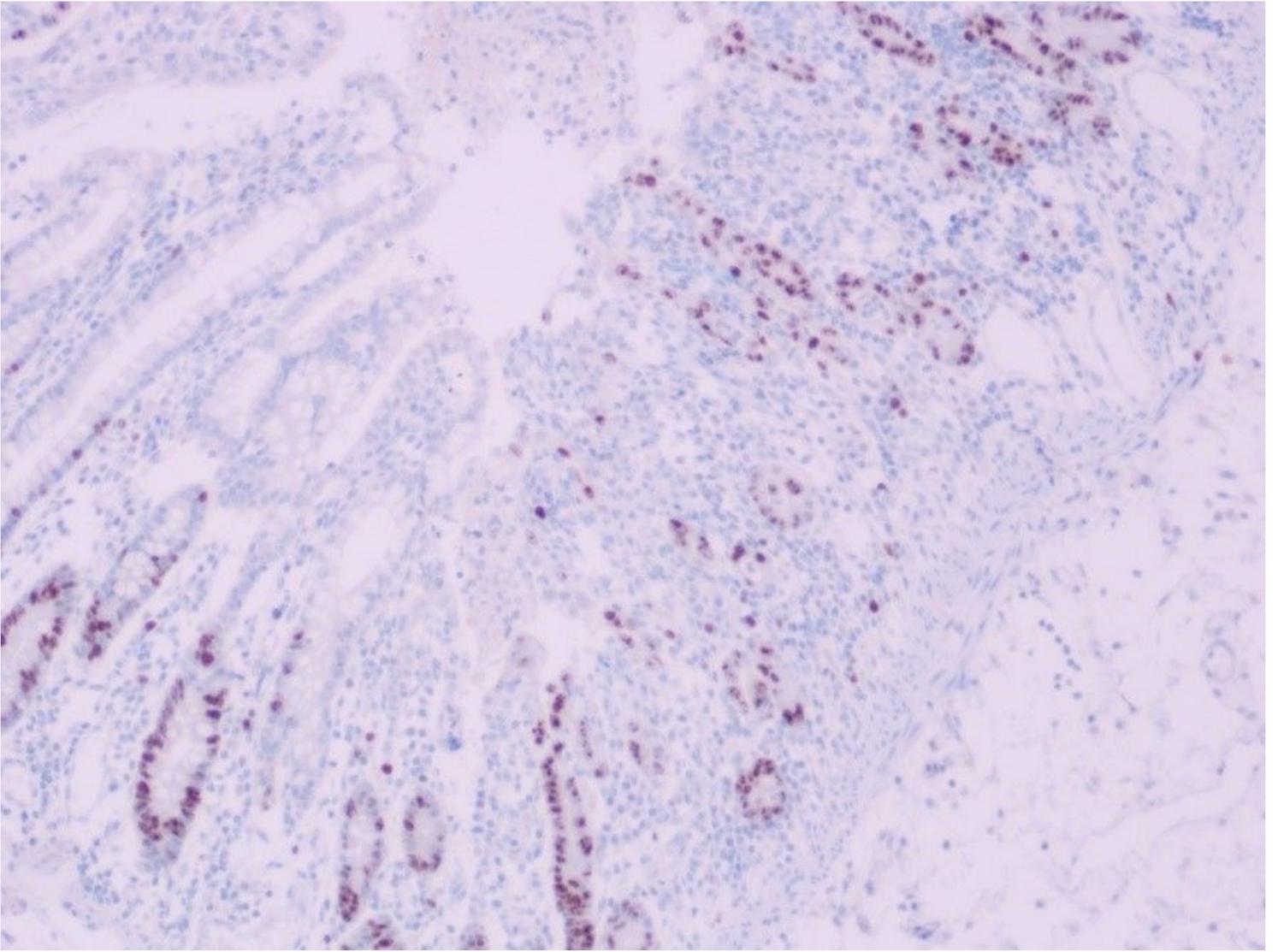
**Figure 12**

Diminish of the normal diffuse staining pattern for E-cadherin in the infected cells (original magnification x100).



**Figure 13**

Aberrant cytoplasmic expression of EMA in a subset of the infected enterocytes (original magnification x100).



**Figure 14**

Expression of Ki-67 labelling index in the infected villi, in contrast to the limited staining of crypts in the normal mucosa at left side of image (original magnification x100).