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CASE REPORT

Neuroendocrine Tumor of the Large Intestine in a Dog

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Abstract

This paper describes the diagnosis and treatment of a 5-year-old Standard Schnauzer dog admitted to the clinic due to a liver failure and chronic bloody diarrhea. Based on the initial examination, no diagnosis could be made, therefore an endoscopic examination of the gastrointestinal tract was performed. The endoscopy revealed a tumor within the ostium *ileocaecocolicum*. Unfortunately, shortly after this procedure, a massive hemorrhage occurred, which led to the animal's death. The necropsy confirmed the presence of the tumor. The histopathological and immunohistochemical results of the collected specimens showed a neuroendocrine tumor (NET G1) of the large intestine. In conclusion, gastrointestinal carcinoids in dogs are relatively rare and often misdiagnosed due to complex or non-specific symptomatology.

Keywords: *Dog, Immunohistochemistry, Large intestine, Neuroendocrine carcinoma*

Bir Köpekte Kalın Bağırsak Nöroendokrin Tümörü

Öz

Bu makale, karaciğer yetmezliği ve kronik kanlı ishal nedeniyle kliniğe kabul edilen 5 yaşındaki bir Standart Schnauzer köpeğinde nöroendokrin tümör teşhisini açıklamaktadır. İlk muayeneye bağlı tanı konulamadığı için köpeğin gastrointestinal sisteminin endoskopik muayenesi yapıldı. Endoskopik muayenede *ostium ileocaecolicum* içerisinde bir tümöre rastlandı. Ne yazık ki, bu işlemden kısa bir süre sonra, hayvanın ölümüne yol açan büyük bir kanama meydana geldi. Otopside tümörün varlığı doğrulandı. Alınan örneklerin histopatolojik ve immünohistokimyasal analizi sonucu, kalın bağırsakta bir nöroendokrin tümör (NET G1) belirlendi. Sonuç olarak, köpeklerde gastrointestinal karsinoidler nispeten nadirdir ve karmaşık veya spesifik olmayan semptomatolojisi nedeniyle sıklıkla yanlış teşhis edilirler.

Anahtar sözcükler: *İmmunohistokimya, Kalın bağırsak, Köpek, Nöroendokrin karsinom*

Introduction

Neuroendocrine tumors (NETs) derived from the diffuse endocrine system cells are rare cancers in both humans and animals, including dogs [1]. In most cases, they are localized in the digestive tract, and over 50% of them are carcinoids. Diagnosis of neuroendocrine tumors, including carcinoid carcinoma, is challenging because of the atypical set of disease symptoms and often the small size of the active tumor, and the difficulty in detecting in medical imaging [2]. In our case, we describe a detailed diagnostic procedure that leads to the diagnosis of the disease, i.e. case history, and clinical examination.

Case History

A miniature Schnauzer male five years old dog was admitted to the Veterinary Office, in June 2017. The dog (weight 11.2 kg) was diagnosed with liver insufficiency and hypothyroidism. Despite the treatment with medicines containing phospholipids, ornithine, silymarin, and the hepatic diet, the results of laboratory tests were outside the reference ranges. The blood tests presented by the owner performed in another veterinary clinic showed the following results: aminotransferase - alanine (ALT 236.3 U/L), aspartate aminotransferase (AST 94 U/L), total protein (TP 76.5 g/L), total bilirubin (BIL) 3.9 µmol/L, urea (UREA

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8.62 mmol/L), free T4 (free thyroxine-fT4) 1.07 ng/dL, cholesterol 7.39 mmol/L. The other biochemical blood parameters did not differ from the reference values. The general condition of the dog was good. The palpation of the liver revealed an enlargement and slight pain. The ultrasound examination (US) showed moderately enlarged liver with mild remodeling features; liver edges rounded without focal changes, gallbladder size around 1 cm, a smooth wall without visible stones and deposits, bile ducts not dilated. The ultrasound image of the rest of the internal organs was normal. The following treatment was introduced: timonacic (Heparegen 100 mg, Jelfa Ltd., Jelenia Gora, Poland) 2 x 0.5 tablet, ursodeoxycholic acid (Ursocam 250 mg, Polfarmex Ltd., Kutno, Poland) 1 x 0.25 tablet, silybin 30% (Hepaxan Dog tablets, Vebiot, Debica, Poland) 1 x 2 tablets, levothyroxine (Euthyrox100 µg, Merck KGaA, Darmstadt, Germany) 1 tablet twice a day and a blood test in 3 weeks.

At the end of April 2018, blood and mucus appeared in the stool. During the clinical examination, the general condition was good, the abdomen slightly taut, in the rectum the feces with an admixture of mucus and traces of blood. Symptomatic treatment, fluid therapy, anti-hemorrhagic treatment, and antibiotic therapy were applied. The day after the examination, the dog's clinical condition improved; after another five days, bloody diarrhea, single vomiting, aversion to water, and food appeared. Additional blood tests were performed, including lipase (reference values) as well as x-ray and control abdominal ultrasound. The radiological examination did not show foreign bodies in the gastrointestinal tract or features indicative of intestinal obstruction. The abdominal US revealed large amounts of gases, with a well-preserved wall layer, gallbladder size about 2.5 cm with the presence of numerous deposits. The wall of the intestine in the area of the *ostium ileo-caecocolicum* showed pathologic thickening of the wall and disturbance of the layered structure on the section of about 15 mm, which suggested proliferative changes; the liver normoechogenic, without focal changes, other organs within the normal range. In the morphological examination of the blood, apart from the increase in the level of leukocytes 24 G/L and thrombocytes 690 G/L, the remaining parameters were within normal limits. The hepatic parameters of ALT and AST were respectively: 324.72 U/L and 92.736 U/L. The drugs used included drotaverine (No-spa 20 mg/mL, Sanofi Aventis Ltd., Warsaw, Poland) - 1 mL subcutaneously, ranitidine (Solvertyl 25 mg/mL, ICN Polfa Ltd., Rzeszow, Poland) - 1 mL subcutaneously, tylosin (Biotyl 50 mg/mL, Biowet Drwalew Ltd., Drwalew, Poland) 1.3 mL subcutaneously seven days, and maropitant citrate (Cerenia10 mg/mL, Zoetis Poland Ltd., Warsaw, Poland) - 1 mL subcutaneously. The pharmacological treatment was continued, additional metronidazole (Metronidazol Polpharma 250 mg, Polpharma, Warsaw, Poland) 2 x 0.5 tablet for seven days was administrated and a stool test for the presence of parasites was performed. The flotation result and *Giardia* spp-antigen (ELISA) were negative. Unfortunately, despite the treatment admixtures of mucus and a small amount of blood were present in the stool; therefore, the endoscopic examination of the gastro-intestinal tract was recommended, and phytomenadione was orally prescribed (Vitacon10 mg, Polfa Warszawa, Warsaw, Poland), 2 x tablet daily.

At the end of June 2018, the morphology showed a decrease in erythrocytes 5.25 T/L (5.5-8.5), a decrease in hemoglobin of 11.4 g/dL, and an increase in thrombocytes of 595 G/L. Over the next days, there was a further decrease in erythrocytes (4.93 T/L), hematocrit (30%), hemoglobin (8.3 g/dL). After ten days, the level of erythrocytes dropped to 3.86 T/L, hematocrit 22.8%, hemoglobin 7 g/dL. Despite the deteriorating blood test results, the dog's clinical condition was better and the occurrence of bleeding from the gastrointestinal tract was much less. Oral preparations with iron and folic acid were introduced, and blood was collected on reticulocytes, B12, iron, folic acid, and blood group determination. The level of reticulocytes was 4.75%, iron 3.30 µg/mL, folic acid 22.4 ng/mL, and vitamin B12 804.0 pg/mL. During the following days, the patient's condition did not change significantly.

At the end of August, an infusion colonoscopy was performed under general anesthesia. In the endoscopic examination, the device was introduced into the ileocecal valve area. At this point, the area of the highly congested and slightly bleeding mucous membrane was found *(Fig. 1)*. Also, tissue samples for histopathological examination were collected from this area. Other sections of the mucous membrane were normal. The image of the examination raised the suspicion of intestinal cancer. The day after the colonoscopy, the dog was in clinically stable condition.

On the fourth day after the procedure, the patient's condition deteriorated suddenly. According to the information

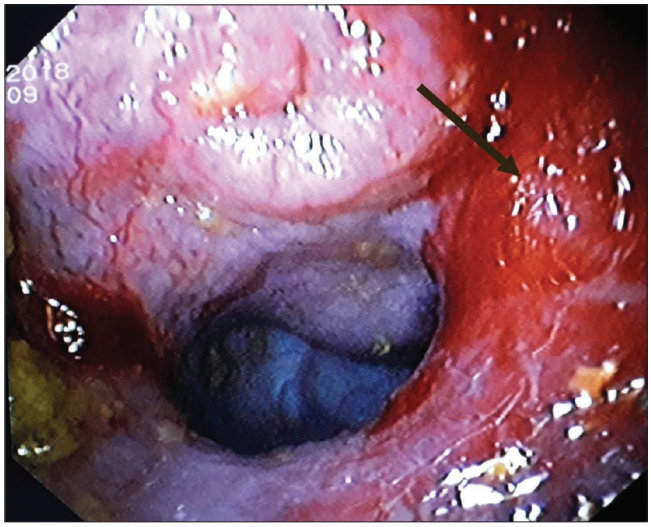

Fig 1. Colonoscopy- inflammation of the mucosa with bleeding in the area of the ostium ileocaecocolicum

obtained from the owner, the dog was very weak, breathed heavily, did not eat or drink, and there was much blood in the feces. In the clinical examination, a very bad general condition was found, conjunctiva and mucous membranes were porcelain white. The stomach was painful palpable, and the spleen strongly enlarged. The dog was administrated 1 mL of dexamethasone (Dexasone 2 mg/mL, ScanVet Poland, Warsaw, Poland) intravenously, and blood was collected for morphology. The blood glucose level was above 600 mg/dL and 5 iu of insulin subcutaneously (Caninsulin 40 iu/mL, MSD Animal Health Poland, Warsaw, Poland) was given. Approximately 30 min after the administration of the drugs, there was an acute cardiorespiratory failure followed by cardiac arrest. Despite the attempted resuscitation, it was not possible to regain vital functions.

We obtained permission from the owners to conduct the necropsy. The examination showed properly developed subcutaneous tissue and muscles, pale red, minor blood extravasation in muscle tissue. A small amount of bloody, clear fluid in the abdomen was present. The digestive tract was gassed, filled with a small amount of mushy food, light brown. In the large intestine, tarry digestive content with visible blood clots was found**.** The necropsy also revealed an enlarged, swollen, and congestive spleen without focal lesions and a thickened, hard fragment of the intestine in the ostium ileocaecocolicum area *(Fig. 2)*. After an incision of the intestinal wall, an extremely altered mucosa with visible ulceration and submucosal hemorrhages, enlarged local lymph nodes, and a structure resembling a neoplastic

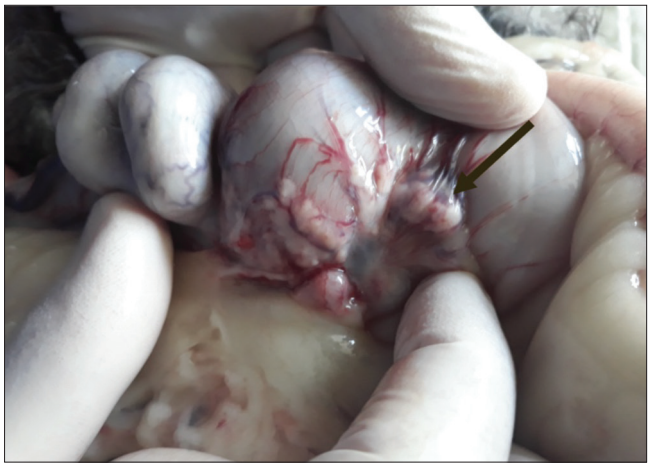

Fig 2. Necropsy - the affected area of the intestine

infiltration were found, the intestinal lumen was filled with blood in this part. The liver was enlarged, firm in consistency, bright red, with no visible nodular changes.

The kidneys were of normal size and shape. The nerve capsule was easily removable, the ratio of the cortex to the medullary layer was maintained. In the chest, a small amount of bloody fluid was found. Lungs were of normal size and shape, aerated, light red. The heart was of normal size and position, with a small amount of clotted blood in the left ventricle. Apart from the primary neoplastic tumor, no macroscopic metastatic changes in the abdominal and thoracic cavity organs were found.

The samples were fixed in a buffered formalin solution and sent to the accredited Laboratory of Oncological Prevention and Diagnostics "Patolog" J&J Głowaccy. The above-mentioned laboratory prepared the preparations using the paraffin technique and performed H&E and mucicarmine staining. The immunohistochemical tests were conducted using Roche's antibodies against synaptophysin, cd 56, ki 67, and chromogranin A, according to the procedures developed by this company *(Table 1)*.

Postmortem samples for histopathological and immunohistochemical examinations were collected.

The result of the histopathological examination of the intestinal wall sections taken in vivo during the endoscopic examination did not give the final diagnosis. In the tissue collected during colonoscopy, the presence of dilated, thrombotic capillaries in the vessels of the lamina propria, and the resulting circulatory disturbances, as well as the accompanying slight lymphatic infiltration were found. The above-described histopathological changes may be the cause of lamina propria bleeding even in minor injuries. The histopathological examination of the specimen taken during the section indicated the presence of carcinoid (neuroendocrine tumor-NET G1) of the large intestine. In this case, the routine H&E staining and immunohistochemistry were performed. The H&E staining revealed the presence of numerous tumor cells in small clusters in the submucosa of the intestinal wall. The microscopic examination revealed small tumor cells, concentrated in clusters, round nucleoli, few figures of the mitotic division *(Fig. 3, Fig. 4)*. There were also single clusters of tumor cells in lymph and capillary vessels *(Fig. 5)*. Staining of synaptophysin *(Fig. 6)* and cd 56 *(Fig. 7)* gave a positive result which confirmed the neuroendocrine activity of tumor cells. Chromogranin A

Table 1. *Panel of primary antibodies used in immunohistochemical techniques*

Antibody	Source	Clone	Dilution	Result
Synaptophysin	Roche	Rabbit Monoclonal Antibody (MRQ-40)	00.04 µg/mL	(+)
Chromogranin A	Roche	Mouse Monoclonal (LK2H10)	1 µg/mL	(-)
CD56	Roche	Rabbit Monoclonal Antibody (MRQ- 42)	0.21 µg/mL	(+)
Ki67	Roche	Rabbit Monoclonal Antibody (30-9)	2 µg/mL	(+)

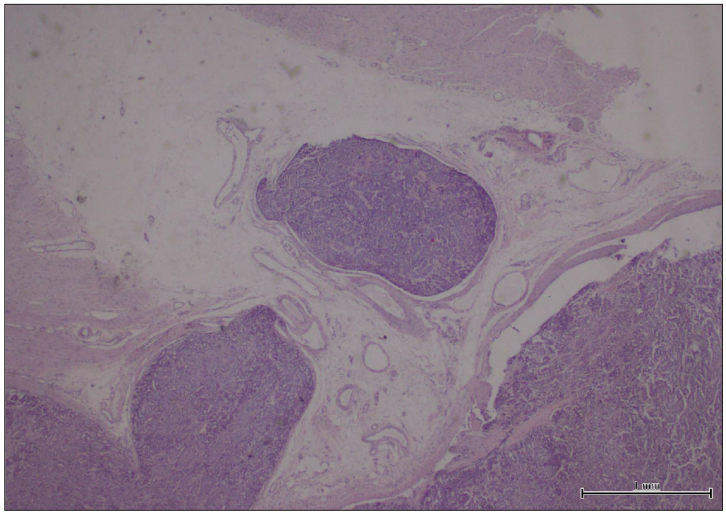

Fig 3. Histological picture of small tumor cells (neuroendocrine tumor - NET G1) of the large intestine. H & E staining. Bar=1 mm

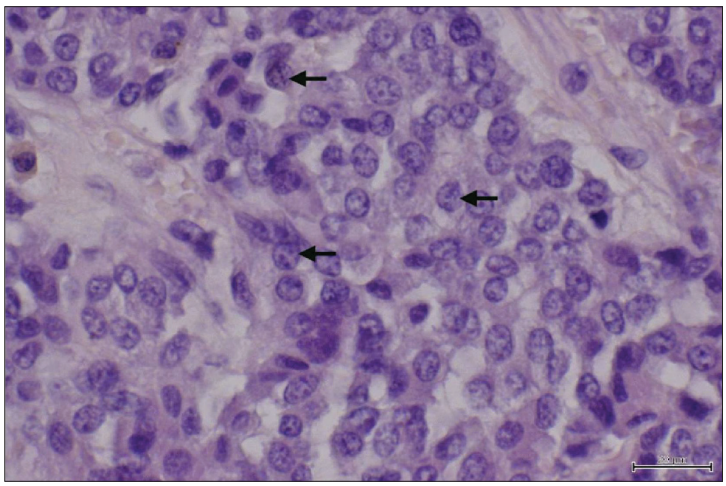

Fig 4. Carcinoma histological image. Small tumor cells with round nucleoli and few figures of mitotic division . Bar=20 µm

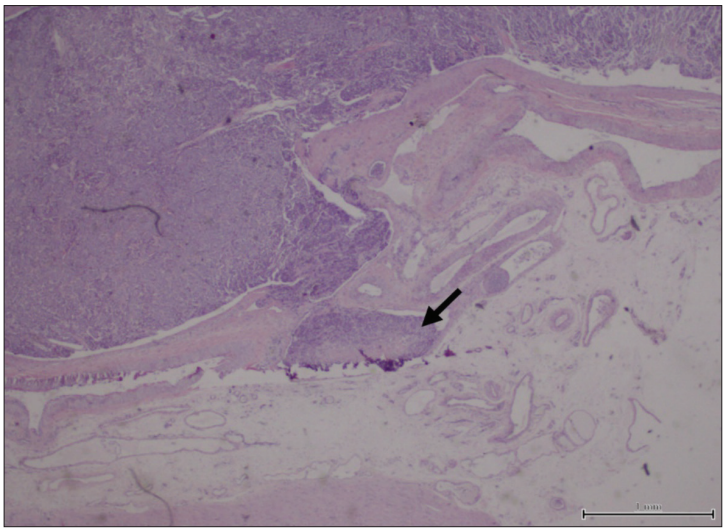

Fig 5. Cancer cells infiltration in lymphatic vessels. H&E staining. Bar=1 mm

staining did not give positive results in both the control sample and tumor cells, which indicates the unsuitability of selected antibodies to staining on dog tissues. Other antibodies should be chosen in this case, or the staining methodology should be modified. However, the positive synaptophysin staining results, and cd 56 were enough to make the diagnosis. In contrast, Ki 67 showed a very low sub-cell index based on which the tumor was classified as NET G1 *(Fig. 8)*. Also, mucicarmine staining was performed to demonstrate the tumor's exocrine capacity and the precise secretion of mucus. This time the staining was negative, which means that the tumor has only endocrine activity and does not produce mucus.

Discussion

Carcinoids pose a diagnostic challenge because they are often innocuous at the time of presentation, requiring a multidisciplinary diagnostic approach with detailed biochemical analysis, cross-sectional and nuclear medicine imaging [3]. Neuroendocrine tumors have rarely been reported in dogs mainly in the intestine, liver, bile duct, lungs, gallbladder, esophagus, skin, and nasal cavity [4]. The most common laboratory techniques used to diagnose the GEP-NEN include the following tests: fasting blood glucose, insulin, and C-peptide concentrations, serum gastrin concentration and gastric pH, serum glucagon concentration, serum VIP concentration, serum chromogranin A concentration, concentration of 5-hydroxyindoleacetic acid in a 24-h urine sample. Tests for chromogranin A and 5-HIAA can give false-positive results for various reasons [5].

A tumor location is crucial for diagnosis and treatment; however, no one technique is sensitive enough; hence, CT (Computed Tomography) or MRI (Magnetic Resonance Imaging) scans should be combined with nuclear medicine imaging [6]. Detection of the tumor and finding its location also created significant problems. It was only at the end of the disease when the tumor was detected in the ultrasound examination, which was then confirmed in the endoscopic examination. Performing CT or MRI scans would have probably helped to diagnose the patient earlier.

However, the final diagnosis was made based on histopathological examinations, in particular, immunohistochemistry performed from collected ex-vivo or post-mortem tissue of the tumor. In addition to the classic H&E staining, to assess the tumor texture and cell structure, immunohistochemical staining (for example, Ki67) allows determining the division capacity of the tumor cells, the so-called

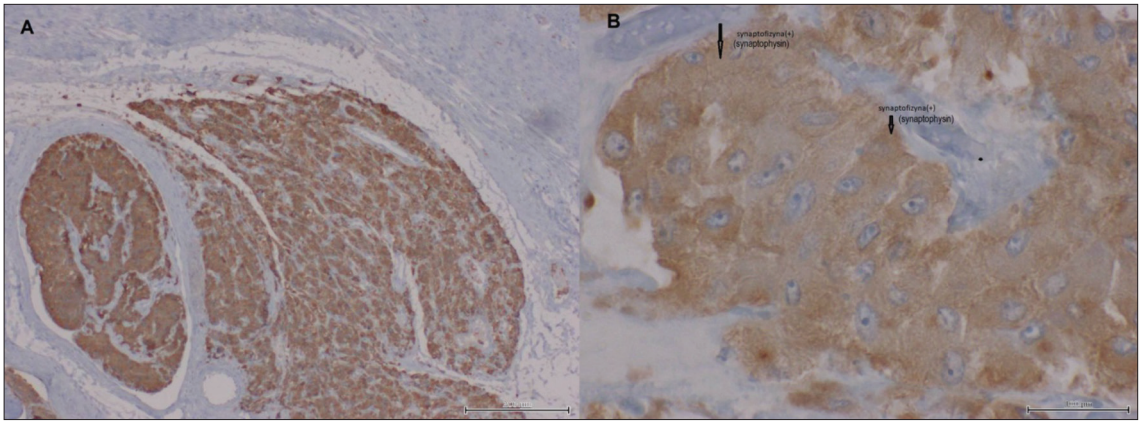

Fig 6. Immunohistological picture of neuroendocrine tumor NET-G1. Positive intracytoplasmic synaptophysin staining. A -Bar=200 µm; B-Bar-=100 µm

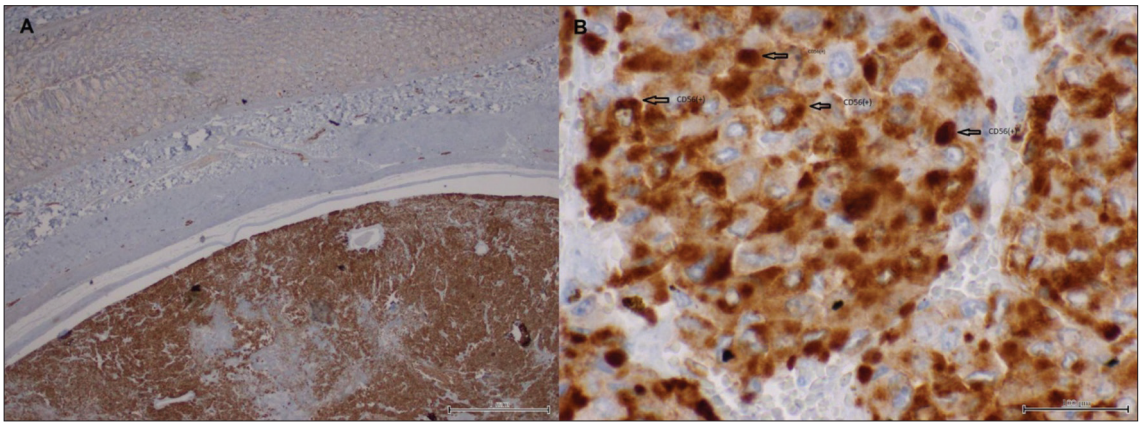

Fig 7. Positive intracytoplasmic CD 56 staining. Single cells within the mucosa. A-Bar=1 mm; B=100 µm

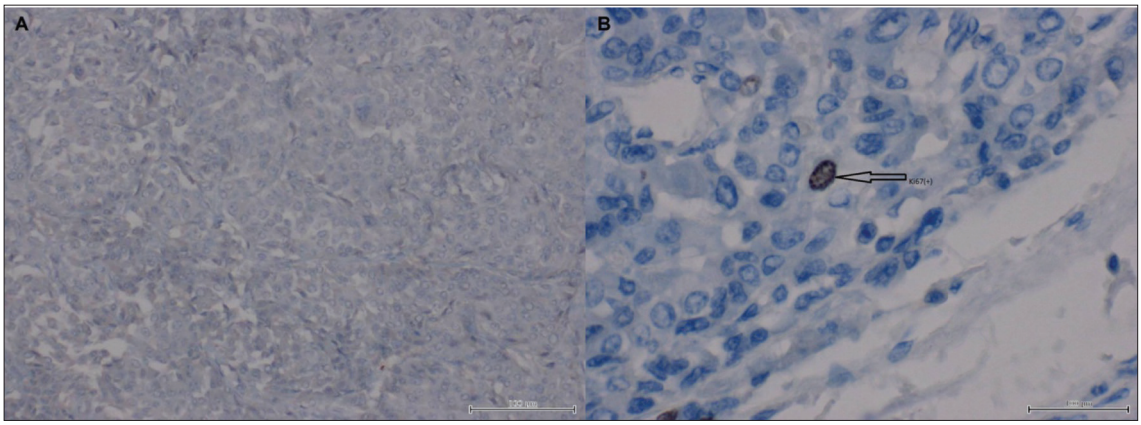

Fig 8. Positive intranuclear Ki-67 staining proliferaion index 3.A-Bar=200 µm ; B=100 µm

mitotic index, which is important in the classification of cell malignancy and their tendency to metastasis. In our case, there was no metastasis observed in other organs. Additional staining, such as synaptophysin, chromogranin A, cd 56, allows demonstrating the neuroendocrine activity of the tumor, which is characteristic for carcinoid cells. The mucicarmine staining for the presence of mucus assesses whether the tumor also has exocrine features. Clinical symptoms depend on the functionality of the tumor. The hormonal activity of tumor cells determines the specific set of symptoms and endocrinological disorders, e.g., insulinoma, glucagonoma, gastrinoma, VIPoma [7]. In the case of our patient, we suspect that chronic liver failure may have been caused by endocrine and biochemical disorders associated with the presence of the tumor. Although non-functioning NETs may secrete hormones [8],

they do not cause any symptoms. Therefore, they are often diagnosed later in the course of the disease with symptoms of metastasis [7].

In conclusion, in our report, we present a neuroendocrine tumor NET, G1 (based on the 2019 WHO Classification) [9] of the gastrointestinal tract in a dog. The extreme difficulty in diagnosing the carcinoid was due to non-specific symptoms, the early onset of the disease, and the limited availability of specialized imaging techniques, including MRI and CT.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contribution

J. Bogucka and S. Slodki conceived and designed the study. S. Slodki collected the data. J. Bogucka analyzed the data. All authors interpreted the data, draft the manuscript, critically revised the manuscript for important intellectual contents, and approved the final version. All authors are in agreement with the content of the manuscript.

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