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Original article

Adenocarcinoma of the posterior segment of the gastrointestinal tract in dogs – clinical, endoscopic, histopathological and immunohistochemical findings

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Abstract

Of all the tumours in dogs, three percent are located in the intestines, and 36-60% of those tumours affect the large intestine. Adenocarcinomas of the intestines account for 20-35% of the gastrointestinal tumours and for almost 60% of the large intestine tumours. The aim of the study was to analyze clinical disorders and endoscopic, histopathological and immunohistochemical changes in colorectal adenocarcinomas in dogs with the use of the E-cadherin, β-catenin, cytokeratin 20 (CK20), Ki-67 and mini-chromosome maintenance 3 (MCM-3). The study comprised 11 dogs of both genders and of different breeds diagnosed with adenocarcinoma of the large intestine. They were from 4 to 11 years old.

The large intestine adenocarcinoma was diagnosed in all the patients. 72.7% cases were diagnosed with a rectal adenocarcinoma, and 27.3% were found to have a colonic adenocarcinoma. All the studied proteins were expressed at different levels and, together with the histological findings, indicated different levels of malignancy (G). The statistical analysis revealed no statistically significant differences between the expression of E-cadherin and β-catenin in the studied tissues ($p=0.79$) and between the expression of Ki-67 and MCM-3 ($p=0.39$). A strong positive correlation was found between the expression of E-cadherin and β-catenin ($r=0.86$; $p<0.05$).

The diagnosis of adenocarcinomas of the large intestine may be facilitated by the introduction of immunohistochemical studies using appropriate cell markers. They may also aid in the accurate evaluation of the biological character of the tumours, their origin, the connections between tumour cells and the mitotic index. That, in turn, may help determine the malignancy and the choice of treatment.

Key words: dog, large intestine, adenocarcinoma, endoscopy, immunohistochemistry

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Introduction

Three percent of all tumours in dogs affect the intestines (Sapierzyński 2006, Morello et al. 2008), and 36-60% of those affect the large intestine (Morello et al. 2008). Tumours of the large intestine are most commonly diagnosed in middle-aged and older dogs, with the highest incidence noted in male dogs between 7 and 9 years old (Patnaik et al. 1977, Terragni et al. 2006, Morello et al. 2008, Doster et al. 2011). German shepherds and collies are considered to be predisposed to tumours of the large intestine (Patnaik et al. 1977, Paoloni et al. 2002, Damasceno et al. 2012). Adenomas are the most common benign tumours, while adenocarcinomas, lymphosarcomas and leiomyosarcomas are the most common malignant tumours of the intestine (Guilford and Strombeck 1996, Morello et al. 2008). Adenocarcinomas of the intestines account for 20-35% of all gastrointestinal tumours and almost 60% of the tumours of the large intestine (Sapierzyński 2006, Morello et al. 2008). The lesions may be pedunculated, infiltrative (diffuse) with an uneven surface and annular. They may protrude into the intestinal lumen or may infiltrate the intestinal wall, leading to a constriction of the lumen (Willard 2005, Sapierzyński 2006, Selting 2013). The clinical signs of neoplastic lesions in the large intestine include the presence of blood in the stool, obstructed defecation, painful defecation and tenesmus (Danova et al. 2006, Terragni et al. 2006, Morello et al. 2008).

Tumours of the large intestine are diagnosed based on the anamnesis, clinical examination and additional tests, such as the complete blood count and biochemistry analysis, an abdominal ultrasound, rectal, endoscopic and histopathological examination (Guilford and Strombeck 1996).

An immunohistochemical (IHC) study using antibodies directed against E-cadherin, β-catenin, cytokeratin 20 (CK20), Ki-67 and the minichromosome maintenance 3 (MCM-3) was planned. Selected cell markers could provide information on the nature of the examined neoplastic lesions.

The E-cadherin and β-catenin that participate in intercellular adhesion may provide information on the possible metastatic potential of the tumour. In addition, an increased expression of Ki-67 and MCM-3, which are markers of cell proliferation, indicates a high mitotic index of the neoplastic cells. Clinically, this points to an increased growth rate and invasiveness of the tumour. The epithelial origin of the adenocarcinoma is confirmed by an increased expression of cytokeratin-20.

The aim of the study was to analyze clinical disorders and endoscopic, histopathological and immunohistochemical changes in colorectal adenocarcinomas in dogs.

Materials and Methods

The study was carried out on 11 dogs of different breeds of both genders. The dogs were from 4 to 11 years old, and were diagnosed with an adenocarcinoma of the large intestine. All the samples were collected from patients of the Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats of the Wroclaw University of Environmental and Life Sciences, whose owners gave consent to participate in the study. Hence, an ethics approval was not necessary. The study was carried out on a small group since adenocarcinomas are rarely diagnosed in the large intestine.

In order to diagnose the gastrointestinal disorder, each dog underwent a clinical examination, a complete blood count and biochemistry analysis, an abdominal ultrasound and a histopathological examination. The endoscopic examination was carried using the Olympus GIF XQ-20 endoscope. Prior to the endoscopy of the terminal part of the gastrointestinal tract, the animals fasted for 24 hours and were given water to drink up to six hours prior to the examination. Three enemas were performed in each animal the day before the endoscopic examination. The endoscopy was carried out under general anesthesia. Xylazine (1 mg/kg m.c.) and atropine (0.05 mg/kg m.c.) were used in premedication and were administered in a single intramuscular injection. Anesthesia was maintained using propofol (initial dose – 4 mg/kg m.c., then to effect) administered intravenously. The biopsies from the macroscopically visible lesions were obtained using the Olympus FB-54K-1 biopsy forceps.

The samples were fixed in 7% buffered formalin for 24 hours. They were then paraffin-embedded and cut into 4 μm sections. The WHO classification was used to histopathologically assess the hematoxylin and eosin stained sections (Kleihues and Sobin 2000).

The IHC analysis was carried out on 4 μm-thick paraffin sections placed on slides (DAKO, Denmark). The sections were then deparaffinised in xylene and passed through a series of decreasing alcohol concentrations to water. The *EnVision™ FLEX Target Retrieval Solution pH 6,0* (DAKO, Denmark) was used to retrieve the antigens from the formalin-fixed tissues. In order to do that, the samples were heated in a 96°C water-bath for 20 minutes. The endogenous peroxidase was blocked using the EnVision™ FLEX Peroxidase-Blocking Reagent for

10 minut. Next, the DAKO:Monoclonal Mouse Anti-Human Mouse Beta-Catenin – clone β-Catenin-1 (diluted at 1:100), Monoclonal Mouse Anti-Human E-Cadherin- clone NCH-38, (diluted 1:50), Monoclonal Mouse Anti-Human Ki-67- clone MIB-1 (diluted at 1:100), Monoclonal Mouse Anti-Human Cytokeratin 20- clone Ks20.8 (diluted at 1:50) and the Novocastra Rabbit Monoclonal Anti-Human MCM-3- clone EP202 (diluted at 1: 50) primary antibodies were applied onto the sections, which were then incubated for 20 minutes at room temperature. Next, the sections were rinsed in the EnVision™ FLEX WashBuffer. The EnVision™ FLEX /HR SM802 visualization system was applied and the slides were incubated for 20 minutes at room temperature. The IHC reaction was visualized using the EnVision™ FLEX DAB+ Chromogen DAKO 3,3diaminobenzidine tetrahydrochloride (DAB) solution. The sections were then rinsed in distilled water. The nuclei were stained with hematoxylin and slides were dehydrated in a series of alcohols. The sections were then cleared in xylene and mounted in a mounting medium Canada balsam.

The photographs of the analysed sections were subjected to a computer-aided image analysis using a computer coupled with an Olympus BX53 (Olympus, Japan) optical microscope and an Olympus Color View IIIu (Olympus, Japan) digital camera. Measurements were taken using the cell^A software (Olympus Soft Imaging Solution GmbH, Germany).

The expression of E-cadherin, β-catenin and cytokeratin 20 was appraised using the modified semiquantitative IRS scale according to Remmele (Remmele and Stegner 1987, Brouckaert et al. 2013). The method takes into account the proportion of positively stained cells and the intensity of the color reaction. The final results represent the influence of both parameters, with values ranging from 0 to 12 points (no reaction = 0 points (-); weak reaction = 1-2 points (+), moderate reaction = 3-4 points (++), intense reaction = 6-12 points (+++)). The expression of Ki-67 and MCM-3 was evaluated quantitatively by estimating the percentage of positive cells (0-5% = no reaction (-), 6-25% = weak reaction (+), 26-50% = moderate reaction (++), above 50% = intense reaction (+++)).

The statistical analysis was carried out using the StatisticaPL for Windows (StatSoft, Poland) software. The data were tested for normality using Shapiro-Wilk test. The expression level of the antibodies was compared using the Wilcoxon test for paired samples. Correlations were assessed using the R Spearman test. Statistical significance was set at $p=0.05$.

Results

Adenocarcinoma was confirmed in all the studied patients based on the histopathological findings. The patients included two boxers (18.2%), two German shepherds (18.2%), two Irish setters (18.2%), one Foxterrier (9.1%), one American Staffordshire terrier (9.1%), one Schnauzer (9.1%), one Bernese mountain dog (9.1%) and one Yorkshire terrier (9.1%). The mean age of the dogs with the neoplastic lesions was 7 years (7.27±1.9). Nine animals were male (81.8%), and two (18.2%) were female. The clinical signs in those dogs included blood in the stool in 11 cases, mucus in stool in three cases, tenesmus in four cases, an eversion of the tumour during defecation in two cases, alternating formed and loose stools in three dogs, loose stool in five dogs, alternating constipation and diarrhea in one dog and normal stool in two dogs. The rectal examination revealed the presence of a hyperplastic lesion with an uneven surface in seven cases. The intestinal lumen was significantly narrowed in two of those cases. In two further cases, the lesion could be everted out of the rectum. Two dogs had an increased leukocyte count (WBC) (dog 1 – 17.5 G/l; dog 2 – 19.0 G/l, reference range: 6-15 G/l); two dogs had a decreased red blood cell count (RBC) (dog 1 – 2.1 T/l; dog 2 – 2.25 T/l, reference range: 5.5-8.9 T/l); two dogs had low hemoglobin concentrations (HGB) (dog 1 – 2.2 mmol/l; dog 2 – 2.3 mmol/l, reference range: 7.4-11.8 mmol/l); two dogs had a low mean corpuscular volume (MCV) (dog 1 – 54 fl; dog 2 – 55 fl, reference range: 60-77 fl); two dogs had low mean corpuscular hemoglobin (MCH) (dog 1 – 1.05 fmol; dog 2 – 1,09 fmol, reference range: 1.18-1.49 fmol) and two dogs had a low mean corpuscular hemoglobin concentration (MCHC) (dog 1 – 19.3 mmol/l, dog 2 – 19.5 mmol/l, reference range: 19.8-22.4 mmol/l). An increased alanine aminotransferase (ALT) serum concentration was present in two cases (dog 1 – 124 U/l; dog 2 – 146 U/l; reference range: < 100 U/l). An increased aspartate aminotrasferase (AST) serum concentration was found in two cases (dog 1 – 105 U/l; dog 2 – 126 U/l; reference range: < 90 U/l). Also, an increased alkaline phosphatase (ALP) concentration was noted in two dogs (dog 1 – 285 U/l; dog 2 – 322 U/l; reference range: < 200 U/l). The remaining parameters were within the reference ranges. A thickening of the colon wall and an enlargement of the regional lymph nodes were visible in the ultrasound examination in three cases. Hyperplastic lesions with an uneven surface and a tendency to bleed were seen in the endoscopic examination in the large intestine in all the cases. The neoplastic lesions were located in the rectum and in the descending colon, in eight and three cases, respectively.

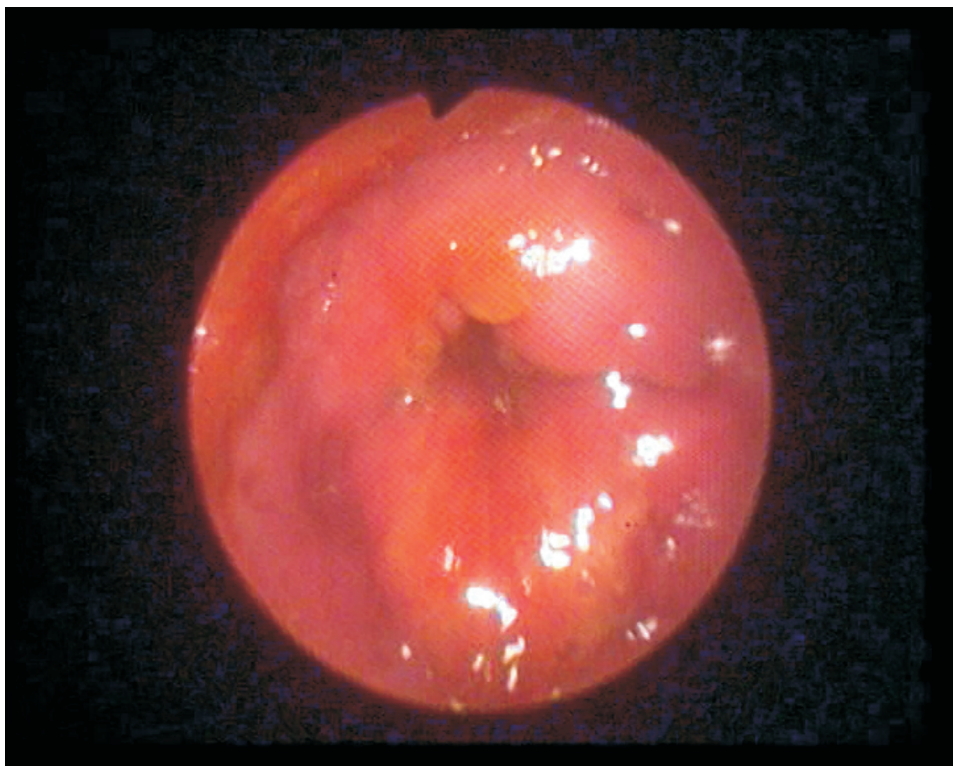

Fig. 1. Neoplastic lesions causing circular narrowing of the rectal lumen – endoscopic image.

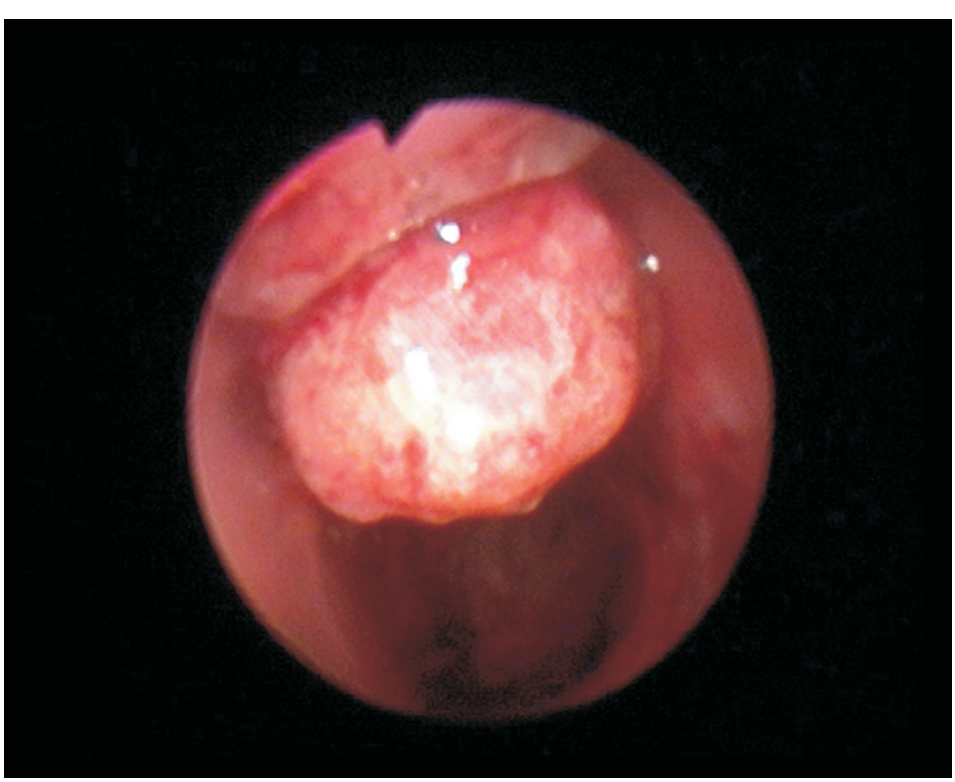

Fig. 2. Neoplastic lesions protruding into the rectal lumen – endoscopic image.

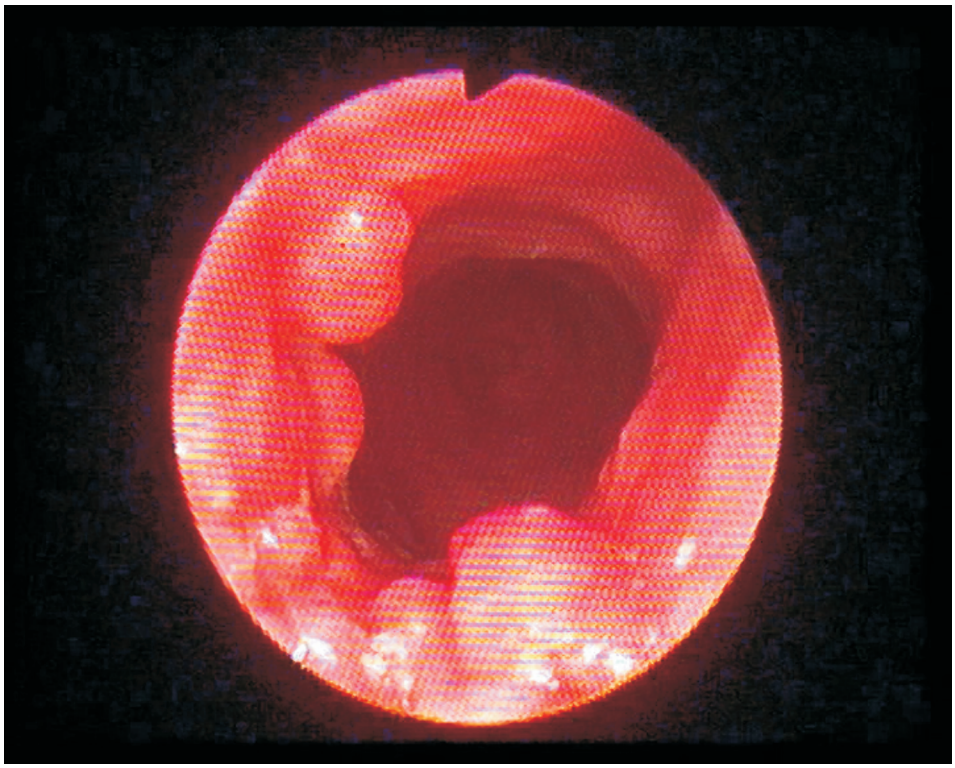

Fig. 3. Numerous small tumours protruding into the rectal lumen – endoscopic image.

The neoplastic lesions infiltrated the intestinal wall in two dogs, causing significant circular narrowing of the lumen. The mucous membrane was visibly thickened in one case (Fig. 1) and had an uneven surface with ulcerations in the second case. In five dogs, the hyperplastic rectal lesions projected into the lumen and caused its narrowing in two cases (Fig. 2). Those lesions had an uneven surface and the mucous membrane was reddened, swollen, and fragile with a tendency to bleed. One dog had numerous small uneven elevations with a reddened, swollen and fragile mucous membrane (Fig. 3).

The hyperplastic lesions in the colon infiltrated the intestinal wall in three dogs and caused luminal circular narrowing. The mucous membrane was thickened, reddened, swollen and fragile. In one case, there were numerous mucous membrane ulcerations at the site of the hyperplastic lesions.

Six of the 11 dogs diagnosed with adenocarcinoma, based on the results of the histopathological examination of the biopsy samples, underwent surgery to remove the lesions. In one dog, the lesion could be everted out of the rectum during the rectal examination. In that dog, the lesion had a fragile consistency bled easily and could be biopsied easily. The lesion was found to be a low grade (G1) adenocarcinoma. The second dog had a hyperplastic lesion with an uneven surface that bled easily, and caused luminal ring narrowing. The tumour was found to be a high grade (G3) adenocarcinoma. The third dog had a single lesion with an uneven surface, which had a fragile consistency and was diagnosed as a moderate-grade adenocarcinoma. In the above cases, the lesions were located in the rectum and they recurred after surgery. The recurrence occurred 14 months after surgery in one dog, six months after surgery in the second dog and four months after surgery in the third dog. In the remaining dogs that underwent surgery, a tumour recurrence did not take place during the two year observation period. In one of those dogs, the lesions were localized in the colon. In two dogs, the lesions were found in the rectum. The lesions in the colon infiltrated the mucous membrane and caused a circular narrowing of the colonic lumen. The lesion was found to be a low grade (G1) adenocarcinoma. The lesions in the rectum had an uneven surface and, in one case, the lesion could be everted out of the rectum during the rectal examination. In both cases, the lesions in the rectum were found to be a low grade (G1) adenocarcinoma. All dogs had chest X-rays, an abdominal ultrasound and a biopsy of the enlarged lymph nodes prior to surgery to exclude metastases.

Of the remaining dogs diagnosed with an adenocarcinoma of the large intestine, one dog was euthanized due to significant defecation problems. The owner of that dog declined surgery due to the localization of the lesion and the surgical risks. The mucous membrane of that dog was thickened and the exten-

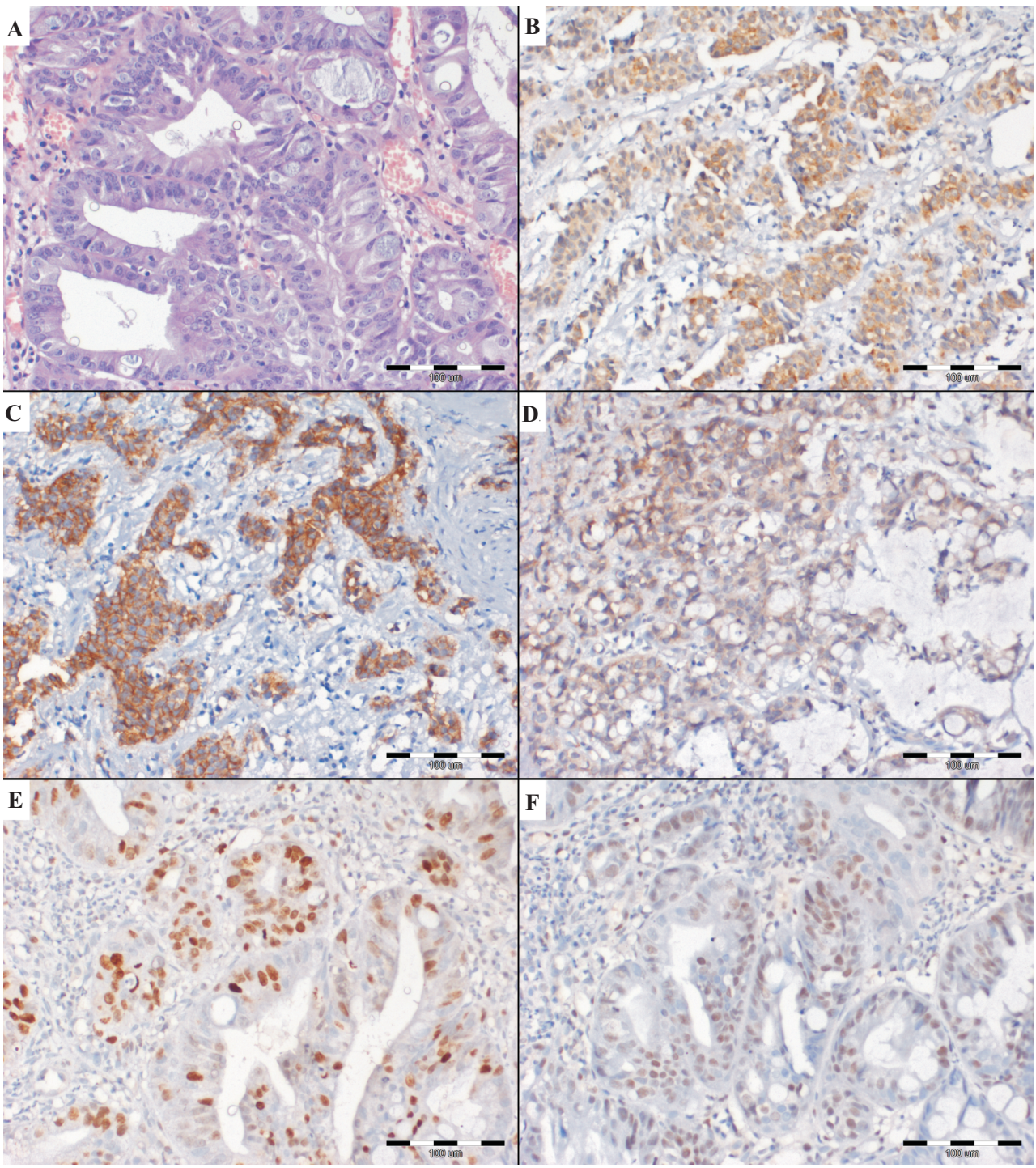

Fig. 4. The histopathological staining and immunoexpression of the selected cell markers in adenocarcinomas of the large intestine in dogs. A – Adenocarcinoma of the large intestine (H&E), B – The expression of cytokeratin in the cytoplasm of the neoplastic cells (IHC), C – The E-cadherin membrane reaction (IHC), D – the cytoplasmic and membrane expression of β-catenin (IHC), E – The nuclear expression of Ki-67 (IHC), F – The nuclear expression of MCM-3 (IHC).

sive neoplastic lesions caused severe narrowing of the rectal lumen. The histopathological examination revealed a high-grade (G3) adenocarcinoma.

The authors are unsure whether the remaining four dogs underwent surgery. The dogs were lost to follow-up after the endoscopic examination and collection of the biopsy samples.

Immunohistochemistry revealed an expression of all the studied proteins. The results are presented in Table 1 and Fig. 4. The expression of the studied proteins with relation to the malignancy degree based on the histopathological examination is presented in Table 2.

Table 1. The expression of chosen markers in the adenocarcinomas of the large intestine in dogs.

Reaction intensity	E-cadherin	β-catenin	cytokeratin 20	MCM-3	Ki-67
			%		
–	9.1	9.1	0	0	0
+	0	0	0	9.1	9.1
++	9.1	9.1	9.1	18.2	27.3
+++	81.8	81.8	90.9	72.7	63.6

Table 2. The expression of chosen markers in the adenocarcinomas of the large intestine in dogs in relation to the levels of malignancy (G) assessed histologically.

Malignancy grade	E-cadherin	β-catenin	cytokeratin 20	MCM-3	Ki-67
G1	+++	+++	+++	+/++	+/++
G2	+++	+++	+++	++	++
G3	+++	+++	+++	+++	+++

The statistical analysis revealed no statistically significant differences between the expression of E-cadherin and β-catenin in the analysed tissues ($p=0.79$) and between the expression of Ki-67 and MCM-3 ($p=0.39$). A strong positive correlation was found between the expression of E-cadherin and β-catenin ($r=0.86$; $p<0.05$). A strong positive correlation was also found between the expression of Ki-67 and the degree of malignancy of the tumours ($r=0.87$; $p<0.05$). No other correlations between the expressions of the studied proteins were noted.

Discussion

We analysed 11 cases of large intestine adenocarcinoma. Rectal adenocarcinomas were diagnosed in 72.7% of the cases and colon adenocarcinomas were found in 27.3% of the cases. Frgelecova et al. (2013) diagnosed epithelial tumours predominantly in the colorectal region (92.6%), where rectal adenocarcinomas constituted 55.6% and colon adenocarcinomas comprised 33.3% of the study group. According to Patnaik et al. (1977), 52.6% of the large intestine adenocarcinomas were localized to the rectum and 47.4% were localized to the colon. Those findings confirm the observation that rectal adenocarcinomas are more common than colonic adenocarcinomas.

The mean age of the studied dogs in this study was seven years. Frgelecova et al. (2013) observed neoplastic lesions in dogs of a similar age (7-8 years old). Adenocarcinomas of the large intestine were reported in slightly older dogs by Doster et al. (2011) (mean age 8.7 years), Terragni et al. (2006) (mean age 8.9 years) and Patnaik et al. (1977) (mean age 9 years). This confirms that large intestine adenocarcinomas usually occur in middle-aged and older dogs.

It is believed that adenocarcinomas of the large intestine are more common in males than females. This was confirmed in our study, where the vast majority of the patients were males (81.8%). Patnaik et al. (1977), Terragni et al. (2006), Morello et al. (2008) and Doster et al. (2011) also noted that neoplastic lesions in the large intestines occurred more commonly in males than females. The cause of the higher incidence of this type of lesion in male dogs remains undetermined.

German shepherds and Collies are reported to be predisposed to intestinal tumours (Patnaik et al. 1977, Paoloni et al. 2002, Damasceno et al. 2012). Other predisposed breeds reported in literature include the Boxer, West Highland white terrier and the Poodle (Neiger 2006, Frgelecova et al. 2013). We found that adenocarcinomas of the large intestine occurred more commonly in the Boxer, German shepherd and Irish setter. However, due to the small patient sample in our study, it is impossible to determine whether those breeds are indeed predisposed to the intestinal tumours. German shepherds and Boxers are predisposed to the chronic inflammatory bowel disease, such as eosinophilic enteritis and histiocytic ulcerative colitis in Boxers (Guilford and Strombeck 1996). Therefore, chronic inflammation of the large intestine in those breeds may predispose them to intestinal neoplasia, causing a higher incidence of intestinal neoplasia. Other authors also found intestinal neoplasia in predisposed breeds. Patnaik et al. (1977) noted that adenocarcinomas in the intestines most commonly occurred in the German shepherd (9 dogs), mixed-breed dogs (9 dogs), Poodles (5 dogs) and the Collie (3 dogs). Terragni et al. (2006) described

four cases of an adenocarcinoma of the large intestine – two in Collies, one in German shepherd and one in mixed-breed dog. However, in the study by Danova et al. (2006), none of the seven dogs were from predisposed breeds.

The blood test findings are unspecific in intestinal neoplasia. Ulcerations and severe bleeding from tumour may cause leukocytosis and mild to moderate hypochromic microcytic anemia (Sapierzyński 2006). We found leukocytosis and hypochromic microcytic anemia in two dogs in our study. In both cases, the lesions infiltrated the intestinal wall, and there were ulcerations of the mucous membrane. The lesions were located in the rectum in one case and in the colon in the other case. Terragni et al. (2006) reported leukocytosis and neutrophilia, hypochromic microcytic anemia or normochromic normocytic anemia in the majority of their patients.

The clinical signs of tumours of the large intestine are associated with the localization and type of lesion. Blood appears in the stool due to strong intestinal inflammation and ulcerations. Pain on defecation and tenesmus are associated with inflammation, a narrowing of the intestinal lumen and difficult passage of stool. In some cases, there is a circular narrowing of the lumen. In the case of severe tenesmus, tumours in the rectum may be everted outward. The above, described clinical signs in patients were also noted in the studies by Danova et al. (2006), Terragni et al. (2006) and Morello et al. (2008). Tumours in the large intestine can be of various forms. In the endoscopic examination, adenocarcinomas may present as narrow-based or wide-based polypoid growths, often accompanied by ulcerations. They may also take the form of more or less extensive ulcerations, protruding lesions, infiltrating lesions and lesions narrowing the intestinal lumen (Willard 2005, Sapierzyński 2006, Selting 2013). We found adenocarcinomas in the form of single lesions protruding into the intestinal lumen in five cases, lesions infiltrating the intestinal wall causing narrowing of the intestinal lumen in five cases, and numerous small lesions in one case.

Several authors have shown that the survival time of dogs with tumours in the large intestine depends on the type of tumour, its nature, surgical intervention and the size of the surgical margins during the resection of the tumour (Leib 2009). In the study by Church et al. (1987), the survival time was shown to be the longest in animals with adenocarcinomas which grew in the form of polyps (mean survival time of 32 months), while the shortest survival time was observed in the cases with the tumour present in the entire luminal circumference (mean survival time of 1.6 months). The surgical removal of the lesions prolonged the survival time by seven to nine months compared to untreated animals. Danova et al. (2006) studied the survival time after a surgical removal of rectal neoplastic lesions in dogs (observation until the animal's death or completion of the study). They reported that there were no relapses in dogs with rectal adenocarcinomas from 21 to 84 months post-surgery. Morello et al. (2008) found that the mean time from surgery to recurrence of the neoplasia was 44.3 months, while the mean survival time was 44.6 months. We did not observe a recurrence of the neoplasia in three dogs during the two-year observation period. In three dogs, there was a recurrence, which occurred 14 months after surgery, six months after surgery and four months after surgery, respectively. In the dogs that had a recurrence after surgery, the primary lesion was localized close to the anal sphincter, making excision of the lesions with adequate resection margins impossible.

An immunohistochemical analysis was performed to determine the biological nature of the lesions. The epithelial origin of the adenocarcinoma is confirmed by an increased expression of cytokeratin-20. This protein is expressed in normal epithelial cells as well as neoplastic cells of an epithelial origin, in pancreatic cancer, stomach cancer and colon cancer in humans (Chu et al. 2000). To date, 20 different polypeptides have been included in the cytokeratin family. Those polypeptides create the intermediate filaments, which make up the cytoskeleton of the cells extending from the surface of the nucleus to the cell membrane (Moll 1998, Moll et al. 1982, 1992). They may be divided into class I, which includes acidic cytokeratins, and class II, containing neutral and alkaline cytokeratins. We found a strong cytoplasmic reaction in all the studied neoplastic lesions in the canine large intestine, indicating an epithelial origin of that tumour. This also points to the usefulness of cytokeratin-20 as a marker of the type and nature of the tumour in dogs. Chu et al. (2000) and Tot (2003) showed the presence of CK20+ cells in the primary and metastatic focus of the colonic adenocarcinoma in humans.

E-cadherin and β-catenin are markers of the presence and strength of intercellular connections. These proteins may be associated with the formation of metastatic foci (Bracke et al. 1996, Iwaya et al. 2003). β-catenin plays a role in the Wnt/ β-catenin pathway. In normal cells, it is limited to the cell membranes. In neoplastic cells, it is most often expressed in the cytoplasm and less often in the nuclei (Haydon et al. 2002, Iwaya et al. 2003, Stein et al. 2011). β-catenin has a positive effect on the synthesis of metalloproteinase 7, which, in turn, may affect the invasiveness and metastatic potential of the tumour (Brabletz et al. 1999, Iwaya et al. 2003). β-catenin connects with E-cadherin, which is a Ca^{2+}- dependent transmem-

brane protein from the cadherin family (Nozawa et al. 2006, Nowak et al. 2007, Miao et al. 2012, Slowinska-Klencka et al. 2012). The presence of weak connections between those two proteins or an absence of those connections is caused by a lack of or a weak membrane expression of E-cadherin and a change in the expression of β-catenin from the cell membrane to the cytoplasm and/or nucleus. This enhances the release of cells from the primary focus and the formation of metastases. Many authors have found this relationship in various neoplasms, such as the osteosarcoma, stomach cancer and breast cancer (Hirohashi 1998, Kashima et al. 1999, Peralta Soler et al. 1999, Ramesh et al. 1999). We found a strong cytoplasmic and membrane expression of β-catenin and a strong membrane expression of E-cadherin. That may indicate the formation of strong intercellular connections within the tumour, thus lowering the capacity to metastasize. However, we found a high expression of those two proteins in some lesions despite their high grade of malignancy based on a histopathological analysis. That, in turn, may suggest that despite the histologically defined high-grade malignancy of the tumours, they were not prone to metastasis. Based on a clinical anamnesis and additional tests, we did not find metastatic foci in the patients in this study. Similar results were obtained by Dorudi et al. (1993), who assessed the expression of E-cadherin in colonic cancer in humans. In turn, Aresu et al. (2010), presented in their studies in, most cases, reduced expression of investigated markers in correlation with decreasing differentiation and increasing staging of cancer. This confirms that the expression of these proteins may decrease with the grade of tumour and can be valuable information about possible opportunities for the formation of metastatic foci.

The Ki-67 proliferation antigen and MCM-3 are the markers of nuclear expression, and may be used to determine the mitotic index. That, in turn, allows an assessment of the intensity of the proliferation of the neoplastic cells (Gerdes et al. 1984, Scotlandi et al. 1995, Musahl et al. 1998, Endl et al. 2001). Ki-67 is expressed in the G_2 and M phases of the cell cycle (Jansson and Sun 1997). As a result, Ki-67 can be used as a marker of extensively proliferating cells that are not in the resting phase. MCM-3 and five other proteins (MCM-2, MCM-4 to -7) are part of a pre-replication complex, whose activity is enhanced in rapidly dividing cells (Giaginis 2009). The three-point expression of those proteins on the Remmele scale indicates that the studied canine large intestine adenocarcinoma is characterized by intense proliferation. When comparing the degree of malignancy based on the histopathological findings with the expression of the proliferative markers, it may be noted that the number of positive cells increases with the G phase. Those markers confirm the mitotic index of the studied tumours denoting their fast growth. Hence, patient care may be planned based on their expression levels. Takano et al. (1996), Kyzer and Gordon (1997), Jansson and Sun (1997) also obtained high expression levels of Ki-67 in the rectal adenocarcinoma in humans. We found that the expression of the MCM-3 cell proliferation marker was similar to the expression of the Ki-67 protein, indicating that it may be useful in the additional assessment of the degree of malignance of this type of tumour in dogs. In a study by Nishihara et al. (2008) on colorectal cancer, the expression of the MCM-2 and MCM-7 minichromosome maintenance complex proteins was evaluated. The results from that study were similar to our findings. Our results indicate that both markers are reliable and useful in determining the proliferative index of the adenocarcinoma of the large intestine in dogs. The results indicate that Ki-67 and MCM-3 are useful and reliable markers of the proliferative index of the adenocarcinoma of the large intestine in dogs. When measured in conjunction with E-cadherin and β-catenin, they may indicate the metastatic potential of the studied tumour.

We found that the majority of the rectal lesions were single, protruded into the intestinal lumen and had an uneven surface. Lesions in the colon were limited to the descending colon. They were infiltrative and caused a circular narrowing of the intestinal lumen. Metastases were not found in other organs and lymph nodes in any of the patients, which may signify a low metastatic potential of the studied adenocarcinomas. The results of immunohistochemical examination of the expression of E-cadherin and β-catenin confirm this finding. The high expression of Ki-67 and MCM-3 points to an increased neoplastic cell proliferation. Hence, such patients need to be closely monitored and immediate surgery should be performed. The choice of treatment of intestinal tumours depends on the type of tumour, the extensiveness of the lesions and the presence of metastases. In the case of adenocarcinomas of the large intestine, surgery is the treatment of choice. It is not always possible to remove the tumour with adequate surgical margins. Therefore, it is crucial to monitor the condition of the patient following surgery. Routine clinical, rectal and endoscopic examinations and abdominal ultrasounds should be performed.

Conclusion

The diagnosis of adenocarcinomas in the large intestine in dogs is time-consuming and requires experience. Correct diagnosis of neoplastic lesions needs to

be based on thorough clinical and endoscopic examinations as well as a histological examination of biopsied samples. Immunohistochemistry, with the use of appropriate cell markers, lets us know more about examined tumour as regards its origin, cell adhesion status or its mitotic index.

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