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Case Report

MULTIPLE OMENTAL HEMANGIOMAS IN A HIMALAYAN CAT: INCIDENTAL FINDING IN A LAPAROTOMY

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Abstract

This report describes the occurrence of omental hemangioma in a five-year-old Himalayan cat. The cat was affected by hemorrhagic gastroenteritis caused by *Fusobacterium necrophorum*. Also, chronic renal failure (CRF) was demonstrated according to high levels of blood urine nitrogen (BUN), creatinine (Cr), as well as hypoproteinemia and anemia. In this respect, in urinalysis, urine specific gravity (USG) decreased while urea, creatinine and total protein levels increased. Moreover, the complete blood count (CBC) tests showed neutrophilia, monocytosis and lymphopenia. During an exploratory laparotomy, nine masses with a size of 1-5 mm and firm consistency were found to be scattered on the omentum. Histologically, the masses consisted of capillary-cavernous vessels with well-differentiated endothelial cells. No mitotic figures, hemorrhage, or necrosis were found, but there was focal lymphocytic infiltration in the parenchyma of the masses. Immunohistochemically, expression of vimentin and von Willebrand factor (vWF) was found in the endothelial cells, while the immunoreaction to smooth muscle actin (αSMA) was negative. These findings confirmed the diagnosis of hemangioma. To the best of the authors' knowledge, this is the first report of feline omental hemangioma.

Key words: cat, hemangioma, immunohistochemistry, omental

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CASE PRESENTATION

A five-year-old intact female Himalayan cat weighing 1.8 kg was referred to the veterinary clinic of Urmia Islamic Azad University, Iran in June 2020. The cat had been dewormed by praziquantel (Alfasan, Netherlands) for four months before it was referred to the clinic. Also, she had been vaccinated with tricat vaccine (against feline panleukopenia virus, feline herpes virus and feline calicivirus) (Biofel PCH, Bioveta Inc., Czech Republic) two months before the visit. This cat presented with lethargy, anorexia, weakness, and melena. Firstly, a complete blood count (CBC) test, differential white blood cell count, and fecal culture were performed according to the routine protocol. A Gram-stained slide was prepared from stool secretions in which rod-shaped Gram-negative bacteria were observed. Next, the secretions were streaked on MacConkey agar (Merck, Germany) and blood agar (Merck, Germany) and incubated at 37 ºC for 48 h. After 24 and 48 h, due to lack of aerobic bacterial growth on the mentioned culture media, anaerobic culture was performed on sterilized *Brucella* agar (Sigma-Aldrich, USA) for 6 days at 37 ºC (Carvallo et al., 2020). Finally, *Fusobacterium necrophorum* was isolated by bacterial culture. According to antibiotic susceptibility test results (Table 1), ceftriaxone (25 mg/kg IM) (Loghman Pharmaceuticals, Iran) was administered every 12 h for one week in order to treat enteritis. The general condition of the cat was relatively good for five months. However, the cat was referred back to the veterinary clinic in November 2020 because of clinical signs such as anorexia, halitosis, and hematemesis. On clinical examination, pale discoloration of the mucosal tissues was found and anemia was suspected. Concerning the clinical signs, we performed another CBC test. Also, to routinely measure the amounts of blood urea nitrogen (BUN) and serum creatinine (sCr), commercial kits (Pars Azmoon Inc., Iran) were used. Moreover, routine urine analysis was performed on a urine sample collected from lateral cystocentesis to measure urine specific gravity (USG) and urea and creatinine levels. The radiographic images prepared in the lateral recumbency position showed an obvious decrease in the size of both kidneys (atrophy) (Figure 1B) in the second referral compared to their larger sizes (Figure 1A) in the first referral. Also, no fluid accumulation was discerned in the radiography. An exploratory laparotomy was carried out. Estrous cycle complications of the cat such as anorexia, insomnia, and lethargy could aggravate our animal's disease conditions. In addition, for the sake of reducing the side effects of anesthetic drugs, we decided to perform concurrent ovariohysterectomy to neuter in the shortest possible time while the cat was anesthetized for laparotomy. This eliminated the need for re-anesthesia and complications from surgery.

To perform exploratory laparotomy, we initially used midazolam (Midazolam™, Richmond Vet Pharma, Argentina, 0.1 mg/kg, intravenously) in combination with dexmedetomidine (dexdor®, Orion Pharma, Finland, 0.005 mg/kg, intravenously) as a pre-anesthetic drug to sedate the cat (Robertson et al., 2018). After 10 min, with the aid of a face mask, anesthesia was induced and maintained through a small animal anesthesia machine with an isoflurane vaporizer and oxygen cylinder (vapored 2%

isoflurane + 98% oxygen) (Drager Sulla 808v, Germany). A 7-cm incision was made through the linea alba to access and inspect the cat's bladder, kidneys, spleen, liver, intestines, and omentum. Also, to avoid bacterial infection, ampicillin (Ampivil®, Daana Pharma co., Iran, 10 mg/kg, intramuscularly) was administered for six days after surgery. Due to about 8% dehydration (moderate loss of skin turgor, dry mucous membranes, weak rapid pulses and enophthalmos) in our cat and based on routine surgery protocol, fluid therapy was performed. Accordingly, before and during surgery, 8 ml/kg/hr and 5 ml/kg/hr of intravenous crystalloid normal saline solution (0.9% NaCl solution), were respectively administered for fluid therapy using one 24 G catheter.

Table 1. Antibiotic susceptibility of *Fusobacterium necrophorum* isolated from the cat's stool secretions by bacterial cultures

Antibiotics	Susceptibility	
	Sensitive	Resistant
Amikacin	_	+
Amoxicillin – Clavulanate	+	-
Ceftriaxone	+	-
Clindamycin	+	-
Chloramphenicol	+	-
Erythromycin	+	-
Gentamicin	-	+
Kanamycin	-	+
Metronidazole	+	-
Nalidixic acid	-	+
Penicillin G	+	-
Streptomycin	-	+
Oxytetracycline	+	-
Vancomycin	-	+

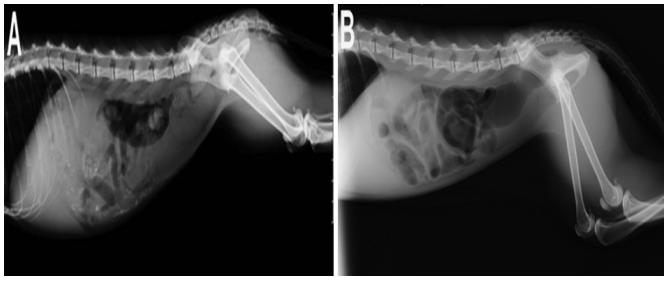

Figure 1. Radiographic images in lateral recumbency position. An obvious decrease in the size of both kidneys (atrophy) **(B)** with specified outlines is seen in the second referral compared larger kidneys **(A)** with specified outlines in the first referral. No fluid accumulation is observed in the radiographs.

During the exploratory surgery, nine 1-5 mm round dark-red solitary masses were found on the omentum (Figures 2A and 2B). Also, no gross abnormalities or changes were seen in the spleen, liver, or intestines while the kidneys displayed a firm consistency with shrunken and pale white discoloration appearance (Figure 2C). The omental masses had a firm and cartilage-like consistency. They were excised from the omentum by Metzenbaum scissors via laparotomy. The collected masses were placed in 10% neutral buffer formalin for fixation. After fixation, tissues were processed by dehydration, clearing, and impregnation. The paraffin blocks were then cut with a rotary microtome (Leica RM2125 RTS) to obtain tissue sections of 6 μm thickness. The sections were stained with hematoxylin and eosin (H&E). Furthermore, immunohistochemical staining was conducted based on the EnVision /HRP system staining method. In brief, the slides were deparaffinized, and to stop the endogenous peroxidase activity, they were incubated with hydrogen peroxide (H_2O_2) (3%) in methanol for 20 min. For antigen retrieval, the sections were microwaved in citrate buffer (pH 6.0) in a microwave oven for 10 min. Thereafter, they were incubated overnight with the primary antibodies at 4 ºC in a humid chamber. The primary antibodies that were used for immunohistochemistry were polyclonal rabbit anti-

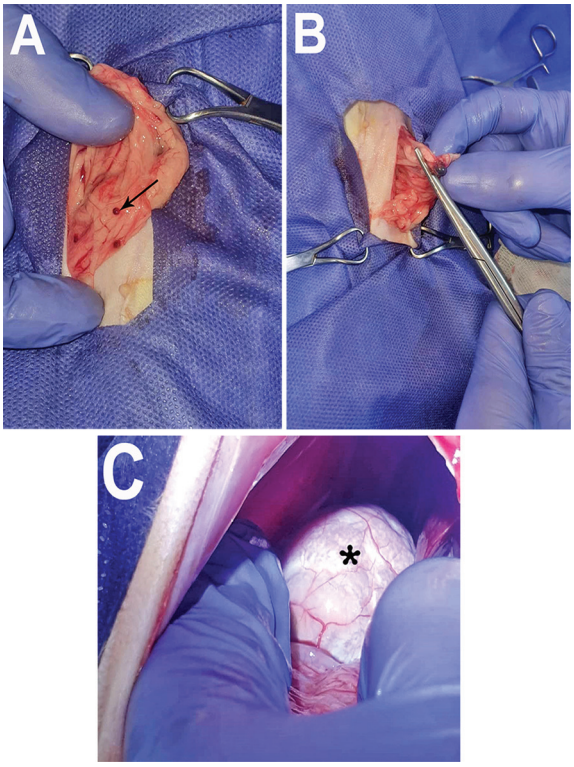

Figure 2. Gross appearance of the omentum and kidney during laparotomic surgery. Hemangiomas are the dark-red masses with a firm consistency on the omentum **(A, B)**. The kidney is shrunken (atrophic) and has a firm consistency with pale-white discoloration **(C)**.

human vWF (dilution 1:500; Dako, USA), monoclonal mouse anti-vimentin antibody (dilution 1:200; Dako, USA), and smooth muscle actin (αSMA) (dilution 1:200; Dako, USA). The primary antibodies were diluted in phosphate-buffered saline (PBS) (pH 7.4, 0.01 M). After the sections were washed in PBS and incubated in a humid chamber, they were coated with the secondary antibody as a labeled polymer with peroxidase (HRP). Subsequently, sections were coated with 3,3'-diaminobenzidine (DAB) plus chromogen and incubated in a humid room for 10 min. Finally, the sections were counterstained with hematoxylin and mounted with mounting media (Amniattalab et al., 2012; Sabattini and Bettini, 2009).

Results of blood biochemical tests for sCr and BUN showed (compared with reference levels) notably elevated levels of sCr and BUN (Table 2). Also, urinalysis revealed a low level of USG and elevated levels of urea, creatinine and total protein compared with reference levels (Table 2).

Table 2. Results of blood and urine biochemical analysis of the cat on second referral to the veterinary clinic in November 2020.

Analysis	Parameter	Result	Reference range
Blood	sCr (µMol/L)	229.8	61.8-185.6
	BUN (mMol/L)	17.9	5.3-11.0
Urine	USG	1.011	1.035-1.045
	Urea (mMol/L)	22	5-10
	Cr (µMol/L)	201	75-180
	TP (g/L)	90	61-86

Abbreviations: sCr, Serum Creatinine; BUN, Blood Urea Nitrogen; USG, Urine Specific Gravity; Cr, Creatinine; TP, Total Protein.

Table 3. Results of blood tests taken from two referrals of the cat to the veterinary clinic.

Test	Result (1)	Result (2)	Reference	Test	Result (1)	Result (2)	Reference
Hct	24.4	21.5	24-45 %	WBC	21.2	23.7	5.5-19.5×10^9/l
Hb	76	68	80-150 g/l	Seg. N.	15.91 (75%)	18.9 (76%)	2.5-12.5×10^9/l
RBC	5.07	4.38	5-10 ×10^{12}/l	Band N.	3.39 (16%)	4.03 (17%)	0-0.3×10^9/l
MCV	42	30	39-55 *f*l	Lymph.	0.63 (3%)	0.23 (1%)	1.5-7×10^9/l
MCHC	31.4	31.5	36-39 g/l	Mono.	1.27 (6%)	1.42 (6%)	0-0.85×10^9/l
PLT	337	130	150-700 ×10^9/l	Eos.	0	0	0.1-1.5×10^9/l
TP	59	54	60-80 g/l	Bas.	0	0	Rare

Note: Result (1), is associated with the first referral of the cat in June 2020. Result (2), is associated with the second referral of the cat in November 2020.
Abbreviations: Hct, Hematocrit; Hb, Hemoglobin; RBC, Red Blood Cells; MCV, Mean Corpuscular Volume; MCHC, Mean Corpuscular Hemoglobin Concentration; PLT, Platelets; TP, Total Protein; WBC, White Blood Cells; Seg. N., Segmented Neutrophil; Band N., Band Neutrophil; Lymph., Lymphocyte; Mono., Monocyte; Eos., Eosinophil; Bas., Basophil.

With respect to this, according to the results of Table 3, as well as the gross appearance of kidneys (Figure 2C), chronic renal failure (CRF) was diagnosed. Indeed, the existence of anemia and hypoproteinemia (Table 3) confirmed the CRF affection. In addition to anemia, the CBC test showed neutrophilia, monocytosis, and lymphopenia in the cat. Accordingly, we advised the cat's owner to use both a low-protein and low-phosphorus diet (Sparkes et al., 2016). On the other hand, a one-month-post-operation monitoring showed that the animal's appetite had improved, halitosis was reduced, and the cat had relatively gained weight.

Histopathological evaluation of the tissue sections of the omental masses showed that their parenchyma was composed of two distinct areas including capillary and cavernous areas with blood-filled vessels (Figure 3A), and the density of the vessels in the capillary area was greater than in the cavernous area. The large cavernous structures had a thick fibrous capsule around them, with a variable number of endothelial cells in central and peripheral areas (Figure 3B), whilst the capillary structures with a papillary pattern of growth were located in a variable amount of connective tissue (Figure 3C). Well-differentiated endothelial cells lining the walls of blood vessels in a single-layer were a fairly uniform spindle to oval in shape. Besides, no necrosis, hemorrhage, or mitotic figures were seen in the masses, although focal lymphocytic inflammation was observed in some of them (Figure 3D). Immunohistochemically, cytoplasmic expression (brown reaction) of vimentin by endothelial cells of the vessels was observed (Figures 4A and 4B), and this expression in capillary areas was more than cavernous areas due to the greater density of endothelial cells in capillary areas of the tumors. In addition, cytoplasmic immunoreaction in vascular endothelial cells to vWF was seen (Figures 4C and 4D). Similar to vimentin, the intensity of vWF expression in capillary areas was greater than in cavernous areas. Overall, we observed positive immunoexpression

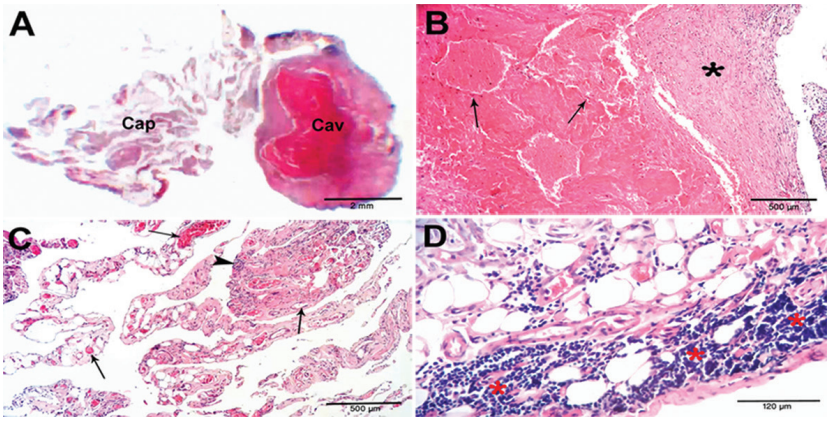

Figure 3. Histopathological findings of feline omental hemangioma. **(A)** The appearance of hemangioma without an objective lens (cav: cavernous area; cap: capillary area). **(B)** Blood-filled cavernous area of the hemangioma with a thick capsule (asterisk) and scattered endothelial cells (arrow). **(C)** The capillary area with the variable-sized capillaries (arrow) and well-differentiated endothelial cells. Focal lymphocytic infiltration (arrowhead) is observed. **(D)** Focal lymphocytic infiltration (red asterisk) around cavernous area. HE.

to both vimentin and vWF in endothelial cells of capillary-cavernous vessels of the tumors, while the intensity of vimentin expression was more than vWF. Also, negative immunoexpression to αSMA was found in vascular structures of the masses. Finally, according to pathological and immunohistochemical characteristics of the masses, a diagnosis of hemangioma was confirmed.

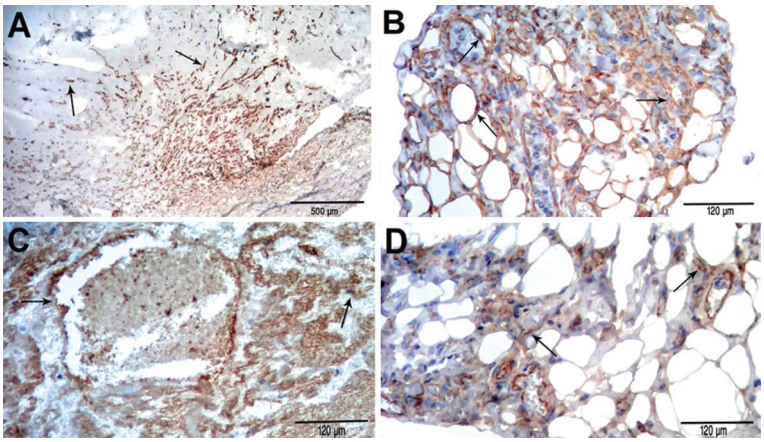

Figure 4. Immunohistochemical labeling of vascular endothelial cells in the capillary-cavernous hemangioma. **(A)** Vimentin expression as a cytoplasmic brown reaction in endothelial cells (arrow) in the cavernous area of the hemangioma. **(B)** Positive immunoreaction to vimentin in endothelial cells (arrow) of the capillary area in the hemangioma. **(C)** Scattered immunoexpression of vWF in endothelial cells of the cavernous area. **(D)** Immunopositivity for vWF in endothelial cells (arrow) of the capillary area in the hemangioma. IHC.

DISCUSSION

Vascular tumors originating from endothelial cells, such as hemangioma and hemangiosarcoma, are rare in cats in comparison with dogs. Generally, these tumors occur in adult cats, so the average affection age has been reported as 10 years (Pirie and Dubielzig, 2006). In this study, the cat was five years old. Her owner stated that he had used a protein-rich diet to feed the animal for one year. Since previous studies have revealed that a high-protein diet in cats is related to inflammatory bowel disease (IBD) caused by *Fusobacterium* (Bermingham et al., 2018), we could suggest that high consumption of protein may have caused an imbalance in intestinal opportunistic microbes and as a result, an increase in *Fusobacterium* had led to hemorrhagic gastroenteritis in the cat (Honneffer et al., 2014). According to Table 3, it seems that thrombocytopenia in the studied cat might have been related to the existence of anemia or loss of blood (hemorrhage) through feces or vomiting (Jordan et al., 1993). On the other hand, according to Table 3, neutrophilia, monocytosis and lymphopenia can be consistent with inflammation. However, a stress mediated component might have contributed as well, given that there was no history of exogenous administration of glucocorticoids

(Tvedten and Raskin, 2012). Furthermore, concurrent monocytosis could be related to a hemorrhagic disease (gastroenteritis). Given the chronic kidney disease in the cat, this leukogram pattern might have been related to CRF as well as nephritis with possible degenerative changes (Tvedten and Raskin, 2012). The case in the present study had CRF, but it was not clear whether this disease was linked to *Fusobacterium*. However, the etiology of hemangiomas are not completely clear, but among animals, it has been postulated that cavernous hemangiomas in rats are associated with infiltration of macrophages, hemorrhage, and severe anemia (Liekens et al., 1999). In cats, viral disease has been suggested as a risk factor for such tumors (Pirie and Dubielzig, 2006). To date, no virus involvement in hemangioma among cats has been reported (Pirie and Dubielzig, 2006). Regarding clinical signs, leukogram profile, radiological findings, and the gross appearance of the abdominal cavity during laparotomy, viral infection cannot be excluded based on the performed diagnostic procedures. The occurrence of hemangioma and hemangiosarcoma are not associated with the breed or sex of cats (Miller et al., 1992). Mainly, surgical excision has been suggested to treat hemangiomas in animals (Miller et al., 1992). Therefore, all of the omental hemangiomas found in the abdominal cavity of the studied case were removed during laparotomy. Hemangiomas are very similar grossly and pathologically to vascular hamartomas. Accordingly, these two vascular lesions should be differentiated by immunohistochemistry (Amniattalab et al., 2012). The immunohistochemical results confirmed the tumor nature of the masses found incidentally on the omentum. Immunoexpression to vimentin and vWF by endothelial cells of capillary-cavernous vessels of these masses, in addition to their histopathological hallmarks as hemangiomas, differentiated them from other tumor-like benign lesions such as vascular hamartomas. Pathological and immunohistochemical findings of the present work are consistent with the results reported by Schoniger et al. (2008). According to these findings, such as the absence of mitosis and pleomorphism in the vascular endothelial cells, as well as the absence of parenchymal hemorrhage and necrosis, we determined that the observed masses were benign. Certainly, vWF is an immunohistochemical marker that helps us diagnose benign or borderline tumors originating from endothelial cells (Amniattalab et al., 2012). Indeed, the positive immunoreaction to both vimentin and vWF in this case revealed the endothelial nature of tumor-forming cells (Zafra et al., 2012). On the other hand, we used αSMA to distinguish hemangioma (true neoplasia) from vascular hamartoma (malformation). The presence of pericytes around the vessels with positive immunoreaction to αSMA was demonstrated in hamartomatous malformation, whereas in the current study, the vascular masses' immunoexpression to αSMA was negative (Martin-Vaquero et al., 2011). Overall, we found multiple feline omental hemangiomas incidentally during laparotomy, and their nature was confirmed as the capillary-cavernous hemangioma and differentiated from similar tumor and tumor-like lesions by pathology and immunohistochemistry. Although the studied cat had hemorrhagic gastroenteritis and CRF, post-operation monitoring showed a relative improvement in the general condition of the animal.

In the present case study, ultrasonographical assessment of the abdomen was not performed in initial examinations. Furthermore, in the one month follow-up only clinical examinations were made, and ultrasonographical assessment of the abdomen and blood works were not performed. These could be considered as limitations of our study.

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Authors' contributions

AA and AR performed surgical procedures and radiography, sampling, biochemical and urine data analysis, pathological and immunohistochemical analysis, manuscript writing and drafting. Both authors read and approved the final manuscript.

Competing interests

The authors confirm that they have no competing interests.

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MULTIPLI OMENTALNI HEMANGIOMI KOD HIMALAJSKE MAČKE: SLUČAJNI NALAZ PRI LAPAROTOMIJI

Amir AMNIATTALAB, Amin REZAZADEH

Kratak sadržaj

Prikaz slučaja opisuje pojavu omentalnog hemangioma kod petogodišnje himalajske mačke. Mačka je imala hemoragični gastroenteritis, izazvan bakterijom *Fusobacterium necrophorum*. Takođe je utvrđena hronična bubrežna insuficijencija na osnovu azotemije, visokih vrednosti kreatinina, kao i hipoproteinemija i anemija. S tim u vezi, specifična težina urina je smanjena, dok su urea, kreatinin i ukupni protein imali visoke vrednosti. Štaviše, kompletna krvna slika je pokazivala neutrofiliju, monocitozu i limfopeniju. Tokom eksplorativne laparotomije, devet masa veličine 1-5 mm, čvrste konzistencije je uočeno na različitim mestima na omentumu. Histološki, mase su se sastojale od kapilarno-kavernoznih sudova, sa dobro diferenciranim endotelnim ćelijama. Nisu pronađene mitotičke figure, krvarenje ili nekroza, dok je u parenhimu masa postojala fokalna limfocitna infiltracija. Imunohistohemijski, ekspresija vimentima i fon

Vilebrandovog faktora (vWF) je utvrđena u endotelnim ćelijama, dok je imunoreakcija na aktin glatkih mišića (αSMA) bila negativna. Ovi nalazi potvrdili su dijagnozu hemangioma. Na osnovu saznanja autora, ovo je prvi izveštaj o omentalnom hemangiomu kod mačaka.

Ključne reči: mačka, hemangiom, imunohistohemija, omentum