RETROSPECTIVE STUDY OF THE EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS OF CUTANEOUS MALIGNANT MESENCHYMAL TUMORS IN DOG

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ABSTRACT

This study evaluated the epidemiological and morphological features, along with the efficiency of the cytological and histopathological diagnosis of cutaneous malignant mesenchymal tumors (MMT) in dogs. The study included 325 dogs with cutaneous MMT presented during five years (2007 – 2011) at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest. It was intended to establish: the predisposition of breed and sex, location of lesions, the incidence by years, seasons and months, and what sampling and type of investigations were used for the diagnosis. During these five years, a total of 3643 dogs with various lesions were subjected to pathological diagnosis and 1262 (34.5%) of them presented cutaneous/subcutaneous masses, of which 325 (25.7%) dogs were diagnosed with MMT. Breed predilection was not detected, but the affected dogs were mainly of medium and large breeds. Sex predilection was not apparent, both sexes being almost equally represented.

The incidence of the cutaneous MMT ranged from 14% in 2009, to 26% in 2011. The median age of the affected dogs increased from 8 years in the first 3 years, to 9 years in the last year of study. About half of the neoplasms were located on the limbs (49%). The attempt to correlate the incidence of the tumors with the season concluded that the majority of the diagnoses were established during spring (20.5%) with the fewest, during summer (12.5%). The samples were obtained by fine needle aspiration (54%) and surgical excision (46%). The most frequent MMT were mast cell tumor, (39%), hemangiopericytoma (24%) and histiocytic tumors (12%).

***Key words**: cutaneous, malignant, mesenchymal, tumors, dog*

MATERIAL AND METHODS

The study included 325 dogs with cutaneous MMT presented during five years (January 2007 – December 2011) at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest.

The main objectives of the study was to determine the incidence of cutaneous MMT, related to age, sex, breed, location and the morphological characteristics of MMT. Other objective of the study was to evaluate the importance of cytology and histopathology in diagnosing of cutaneous lesions.

The cytological samples were collected by fine needle aspiration (FNA) or surgical excision (SE). Impression smears and/or scraping were made from the surgical samples, followed by May–Grünwald Giesma (MGG) and Diff-Quick staining. The surgical excisions were fixed in 10% formaldehyde solution and Bouin's fixative and routinely processed. The sections were cut at 4-6 microns and stained with trichrome Masson or hematoxylin eosine (HE).

RESULTS AND DISSCUTIONS

A total number of 3643 dogs were presented at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest, between January 1st, 2007 and December 31, 2011. A number of 1262 (34,6%) were diagnosed with cutaneous lesions, which brings cutaneous pathology of dog on the first position in our department.

Out of the 1262 dogs with cutaneous lesions, 325 (25.7%) were diagnosed with MMT. These 325 dogs were evaluated for the incidence of MMT related to breed, sex and location and the morphology of MMT. A special consideration was give also to the method of sample collection and examination. The possible correlation between epidemiology and morphology was evaluated by relating the epidemiological data to every year, month and season of the study.

Out of the 500 dogs examined in 2007, 172 (34.4%) had cutaneous lesions, and 55 (32%) of these 172 dogs, had MMT.

Table 1. Cases of cutaneous MMT diagnosed in 2007

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINTION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	7	5F/2M	11 (9-13)	1	0	2	4	4	3	4	0	3
FEB	6	4F/2M	10 (9-12)	0	0	2	4	5	1	5	1	0
MAR	2	2F/0M	9 (5-13)	0	0	1	1	1	1	1	0	1
APR	8	6F/2M	9 (6-12)	1	1	3	5	6	2	6	0	2
MAY	6	3M/3F	10 (6-12)	0	1	2	3	3	3	4	1	1
JUN	3	3M/0F	8 (7-10)	1	1	3	1	2	1	2	0	1
JUL	4	3F/1M	5½ (4-7)	1	0	2	2	3	1	3	0	1
AUG	3	2F/1M	12 (11-13)	0	0	1	2	1	2	2	0	1
SEPT	5	3F/2M	5 (3mon-11)	1	0	2	2	1	4	2	0	3
OCT	7	4F/3M	8 (4,5-12)	2	0	1	4	4	3	4	0	3
NOV	2	2F/0M	10 (8-12)	0	0	2	1	1	1	1	1	0
DEC	2	2F/0M	9 (8-10)	0	0	0	2	0	2	1	0	1
TOTAL	**55**	**39F/16M**	**8 (3mon-13)**	**7**	**3**	**21**	**31**	**31**	**24**	**35**	**3**	**17**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

A number of 39 (71%) were females and 16 (29%) were males. This data contradicts data reported in previous studies, where males were more affected, with a raport of 2:1. The mean age of the dogs in this study was 8 years (range, 3 months – 13 years), is in agreement with other studies.

The cutaneous MMT were located mainly on the trunk and limbs, followed by head and neck. Some dogs had multiple lesions, located in two or three body regions. FNA was used in 30 cases (56.4%) and 25 of these were SEs. Of these 25 cases, 5 (20%) were submitted only to cytological examination, 3 (12%) were submitted only to histopathological examination and 17 (68%) cases were submitted both to cytological and histopathological examination.

Out of the 735 dogs examined in 2008, 277 (37.7%) had cutaneous lesions, and 66 (23.8%) of these 277 dogs, had MMT. Out of the 66 dogs with MMT, 31 (47%) were females and 35 (53%) were males. The mean age was 8 years (range, 3 months – 16 years), similar to the previous year.

Table 2. Cases of cutaneous MMT diagnosed in 2008

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	5	4F/1M	10 (6-16)	1	0	3	2	2	3	3	0	2
FEB	7	3F/4M	9 (5-15)	1	1	3	2	4	3	5	0	2
MAR	7	0F/7M	9,5 (4,5-13)	2	1	2	2	5	2	6	1	0
APR	8	3F/5M	8 (1-13)	5	0	3	2	5	3	5	1	2
MAY	8	5F/3M	8 (9 mon-13)	1	0	2	6	5	3	6	0	2
JUN	9	6F/3M	8 (1-14)	0	0	5	4	5	4	5	3	1
JUL	4	3F/1M	4 (5 mon-9)	0	2	1	3	1	3	3	1	0
AUG	0	0F/0M	-	0	0	0	0	0	0	0	0	0
SEPT	1	1F/0M	8	0	0	0	1	0	1	0	1	0
OCT	6	2F/4M	8 (3mon-13)	0	1	1	6	5	1	6	0	0
NOV	7	2F/5M	10 (7-12)	0	0	3	4	3	4	3	2	2
DEC	4	2F/2M	9 (9-11)	1	0	0	3	3	1	3	1	0
TOTAL	**66**	**31F/ 35M**	**8 (3mon-16)**	**11**	**5**	**20**	**35**	**38**	**28**	**45**	**10**	**11**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

The limbs were the most affected, followed by trunk and head. The neck was the least affected, less than 10% of the lesions had this location. FNA was used in 57.5% (n=38) cases and 42.5% (n=28) of these were SEs. Of these 28 cases, 7 (25%) were submitted only to cytological examination, 10 (35.7%) were submitted only to histopathological examination and 11 (62.7%) cases were submitted both to cytological and histopathological examination. Out of the 700 dogs examined in 2009, 218 (31%) had cutaneous lesions, and 48 (22%) of these 218 dogs, had TMM.

Table 3. Cases of cutaneous TMM diagnosed in 2009

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCTION**				**SAMPLING METHOD**		**EXAMINTION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	3	0F/3M	7½ (2-13)	0	0	3	0	1	2	1	0	2
FEB	5	0F/5M	9 (1-12)	0	0	4	2	2	3	3	0	2
MAR	3	1F/2M	7½ (1-11)	1	0	3	1	2	1	3	0	0
APR	4	3F/1M	7 (5mon-10)	0	0	3	2	4	1	3	0	1
MAI	9	2F/7M	6 (5mon-15)	5	0	3	4	8	1	9	0	0
JUN	4	2F/2M	12 (9-15,5)	0	0	0	4	3	1	3	0	1
JUL	3	2F/1M	10 (9-11)	0	0	2	1	1	2	1	0	2
AUG	1	1F/0M	13	0	0	0	1	1	0	1	0	0
SEPT	2	1F/1M	10 (6-14)	0	0	3	1	2	0	2	0	0
OCT	4	2F/2M	9 (5-12)	0	0	0	4	2	2	3	0	1
NOV	9	4F/5M	9 (1-14)	3	1	6	4	7	2	8	0	1
DEC	1	0F/1M	7	0	0	0	1	0	1	0	0	1
TOTAL	**48**	**18F/30M**	**8 (5mon-15)**	**9**	**1**	**27**	**25**	**32**	**16**	**37**	**0**	**11**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2009, cutaneous MMT affected significantly the males (62.5%, n=30), in comparison with the females (37.5%; n=18). The cutaneous MMT were located mainly on the trunk and limbs. FNA was used in 66.6% (n=32)

cases and 31.0% (n=16) of these were SEs. Of these 16 cases, 5 (31%) were submitted only to cytological examination and 11 (69%) cases were submitted both to cytological and histopathological examination. No sample was submitted only to histopathological examination. Out of the 807 dogs examined in 2010, 236 (29.5%) had cutaneous lesions, and 70 (29.6%) of these 236 dogs, had MMT.

Table 4. Cases of cutaneous MMT diagnosed in 2010

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCTION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	2	0F/2M	7 (3-9)	0	2	2	1	2	0	2	0	0
FEB	5	3F/2M	11 (7-13,5)	1	0	1	3	4	1	4	0	1
MAR	11	7F/4M	8 (3mon-13)	3	0	4	6	5	6	6	0	5
APR	8	6F/2M	10,5 (7-14)	0	0	2	6	4	4	5	0	3
MAY	9	4F/5M	8 (1-14)	1	0	3	6	6	4	6	1	2
JUN	2	2F/0M	8 (4-12)	0	0	1	2	1	1	1	0	1
JUL	1	0F/1M	9	0	0	0	1	1	0	1	0	0
AUG	5	3F/2M	11 (9-12)	1	0	2	2	1	4	4	1	0
SEPT	4	0F/4M	8 (6mon-10)	2	0	1	1	2	2	2	0	2
OCT	8	2F/6M	7,5 (1-13)	0	1	4	5	3	5	4	1	3
NOV	9	4F/5M	8 (2mon-14)	3	1	3	3	3	6	5	2	2
DEC	6	2F/4M	5 (1mon-10)	2	1	1	3	2	4	5	0	1
TOTAL	**70**	**33F/ 37M**	**8½ (1mon-14)**	**13**	**5**	**24**	**39**	**33**	**37**	**45**	**5**	**20**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2010, the number of males (n=37) was almost equal to the number of females (n=33), and the mean age increased with approximately 6 months. There were more cases of MMT located on the head, but the trunk and limbs remained the most affected regions.
In the previous year, FNA was the dominant method of sampling, but this year, FNA and SE were used almost in the same proportion.

Cytological examination remained the main method of examination, but the number of cases submitted both to cytological and histopathological examination increased. The main reason for this could be the interest of clinicians to receive both a fast preliminary diagnosis (cytology), but also a final diagnosis (histopathology). Another reason could be the increase in the level of responsibility of the dog owners, who acknowledge the major role of a final diagnosis in the prognosis and management of the therapy.
Out of the 901 dogs examined in 2011, 359 (39.8%) had cutaneous lesions, and 86 (23.9%) of these 359 dogs, had MMT.

Table 5: Cases of cutaneous TMM diagnosed in 2011

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	4	3F/1M	9 (1-14)	1	0	1	2	1	3	2	0	2
FEB	5	3F/2M	9 (2-12)	3	0	2	1	2	3	2	1	2
MAR	7	4F/3M	9 (2,5-13)	2	0	2	2	3	4	4	1	2
APR	6	2F/4M	3½ (4mon-7)	2	1	2	3	3	3	4	0	2
MAI	8	1F/7M	10(6mon-15)	0	0	3	5	6	2	7	0	1
JUN	11	5F/6M	11 (6-15)	3	0	3	5	1	10	5	1	5
JUL	7	3F/4M	10 (7-11)	0	1	2	4	4	3	5	1	1
AUG	6	4F/2M	9 (5-11)	2	0	3	2	4	2	5	1	0
SEPT	9	3F/6M	8½ (4-12)	1	2	2	5	5	4	5	1	3
OCT	10	6F/4M	10 (7-14)	2	2	4	2	4	6	5	4	1
NOV	5	2F/3M	10 (4-14)	1	2	2	1	4	1	4	1	0
DEC	8	3F/5M	8½ (6-14)	1	0	3	4	5	3	6	2	0
TOTAL	**86**	**39F/ 47M**	**9 (4 mon-15)**	**18**	**8**	**28**	**36**	**42**	**44**	**54**	**13**	**19**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2011, the males (54.6%) were more affected than the females (45.4%). The mean age increased to 9 years, but the age range remained the same. The incidence of the lesions located on the head increased; 21% of the MMTs developed in this location. 50% of the samples were collected by FNA, and 50% were collected by SE. Of these 44 SEs, 12 (27%) were submitted only to cytological examination, 13 (29%) were submitted only to histopathological examination and 19 (44%) cases were submitted both to cytological and histopathological examination. There was a constant and significant increase in the number of cases presented for diagnostic pathology. Thus, in 2011 there was a 80% increase in the number of cases, compared to 2007.

The number of cases with cutaneous lesions in 2011 increased with 90%, compared to 2007. The number of cases of cutaneous MMT increased in 2008, compared to 2007, but decreased in 2009. In 2010 and 2011, there was a constant and significant increase in the number of the cases with MMT. The reason of the 80% increase of the number of cases with cutaneous lesions in 2008, compared with 2007, could be the heat wave in the summer, when the temperature rised above 35°C for long periods of time. The dogs with long hair were more represented, knowing their sensitivity to the heat. The highest and the lowest number of cases with cutaneous MMT were diagnosed during spring and summer, respectively. Possible explanations could be the higher incidence of parasitic infestations (fleas, ticks, and mosquitoes) and allergies, the damaging effects of UV radiations and high temperatures during spring (Cranganu, 2009). The small number of cases in the summer could be correlated with the vacation time of the dog owners and the shorter working hours in our department.

The mean age of the dogs with MMT remained constant in the first 3 years of the study (8 years, range 3 months – 16 years), and increased in 2010 (8.5 years) and 2011 (9 years). The age ranges remained the same, during the 5 years study, but there were an increase in the 9-10 years category.

No breed was overrepresented and the breeds of the dogs in the study were German Shepherd, Boxer, Rottweiler, Labrador, cross-breed and mixed-breed. The breed distribution could be explained by preference of Romanian dog owners for these breeds. The over representation of the large breeds in this study is in agreement with the data reported in previous studies.

FNA was predominantly used between 2007 and 2009 and in the following two years, but the number of SEs increased. Even though histopathological examination is more expensive, it is more reliable in the final diagnosis of the lesion. Cytological examination was the main examination method.

The most frequent MMT were mast cell tumor (MCT) and hemangiopericytoma, followed by histiocytic proliferative disorders and fibrosarcoma. There were infrequent cases of liposarcoma, hemangiosarcoma and myxoma. The type and incidence of cutaneous MMT, according to every year of the study can be found in Table 6.

Tabel 6. The incidence major categories of MMT

Year	***Fibro***	***MFH***	***Lipo***	***Hem***	***HPD***	***MCT***	***Hemperi***	***Other***	***Total***
2007	9	3	0	2	2	26	10	3	**55**
2008	6	3	2	1	5	29	18	3	**66**
2009	8	0	3	0	3	14	17	3	**48**
2010	2	4	3	1	13	29	15	3	**70**
2011	8	1	0	1	17	30	19	9	**86**
Total	**33**	**11**	**8**	**5**	**40**	**128**	**79**	**21**	**325**

Fibro – fibrosarcoma; MFH – malignant fibrous histiocytoma; Lipo – liposarcoma; Hem – hemangiosarcoma; HPD – histiocytic proliferative disorders MCT – mast cell tumors Hemperi – hemangiopericytomas Other – other MMT

In 2009 and 2007, MCT had the highest incidence (29% and 48%, respectively). The incidence of hemangiopericytoma was approximately constant, but it increased in 2009 (35%). Between 2007 and 2009, there were 2-4 (4-8%) HPDs, but their incidence increased to 13 (19%) and 17 (20%) in 2010 and 2011, respectively. The incidence of HPDs increased significantly. The incidence of fibrosarcoma fluctuated from 16% in 2007, to 9% in 2008 and 17% in 2009. Few cases of MFH and hemangiosarcoma were diagnosed, their incidence ranging from 1 to 6%.

The cytological and histopathological aspects of the main MMTs are presented, as follows.

The diagnosis of MCT by cytology is in most of the cases straight forward. The smear is often highly cellular; the cells have round shape and metachromatic cytoplasmic granules. The nuclei are predominantly round and 1 or 2 nucleoli are evident. The cells could show heavy granulation of the cytoplasm, which obscures the nucleus or extended degranulation. The

background contains numerous free granules. The MCTs are rich in fibrous tissue, thus the cytological smear contains fibrocytes and fibroblasts (Fig. 1) The MCTs have various histopathological aspects (Weiss, 1994, Baba, 2002). The neoplastic cells show various grades of anisocytosis and anisokaryosis and are arranged in cords and sheets (Meuten, 2002, Manolescu, 2009). The arrangement of the cells between collagen bundles results in the multilocular appearance of the tumor (Fig. 2).

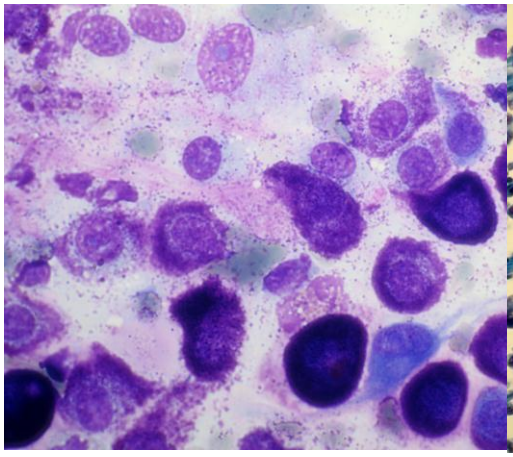

Figure 1. MCT. Highly cellular smear. Round cells with metachromatic cytoplamic granules. M-G.G. stain. x100

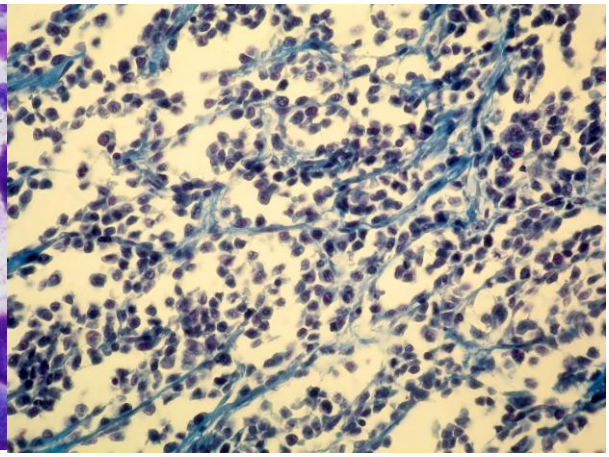

Figure 2. MCT. The cells are arranged in cords, between fibrous bundles. HEA stain. x 40

Hemangiopericytoma is included in the category of spindle cell tumors (Hendrick, 1998). The smears contains a monomorphous population of isolated or aggregated cells with elongated nuclei, 1-2 nucleoli and cytoplasm with short projection or star shaped, which is slightly basophilic (Fig. 3). The majority of the cells are dysplastic or slightly anaplasic.

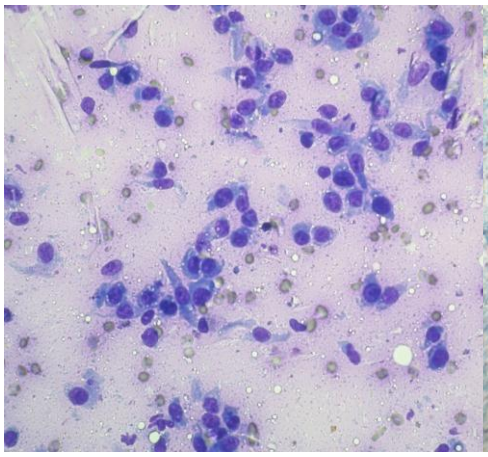

Figure 3. Hemangiopericytoma. Highly cellular smear. Spindle cells, isolated or aggregated, with cytoplasm with short projections or star shaped. M-G.G. stain. x40

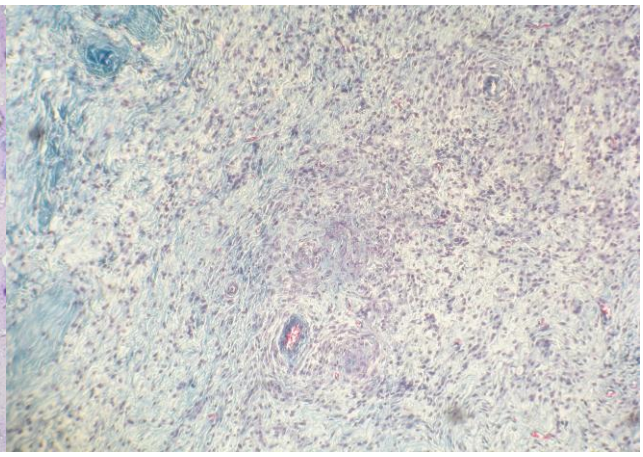

Figure 4. Hemangiopericytoma. Whirls of spindle cells arranged around blood vessels, which results in the "fingerprint" pattern of the tumor. HE stain, x10

The histopathological aspects are characteristic (Goldschmidt, 1992, Meuten, 2002). The spindle cells arearranged in whirls around blood vessels. The "fingerprint" pattern of the tumor can be seen in Fig. 4. The cells proliferated around collapsed capillaries (Baker, 2001, Raskin, 2010).
The cytology of ***fibrosarcoma*** depends on the grade of malignancy of the tumor. In low grades of malignancy, the spindle cells are dysplastic and/or with low anisocytosis and anisokaryosis. In high grade of malignancy, the pleomorphism is more obvious. The isolated cells have oval euchromatic nuclei, evident nucleoli, anizocaryosis and anisocytosis (Fig. 5).

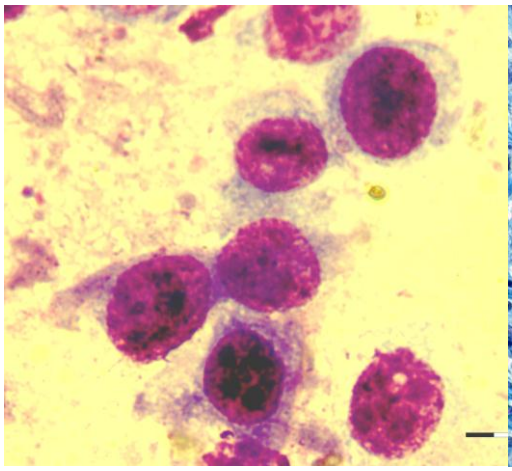

Figure 5. Fibrosarcoma. Monomorphous highly pleomorphic and malignant spindle cells. M-G.G. stain. x100

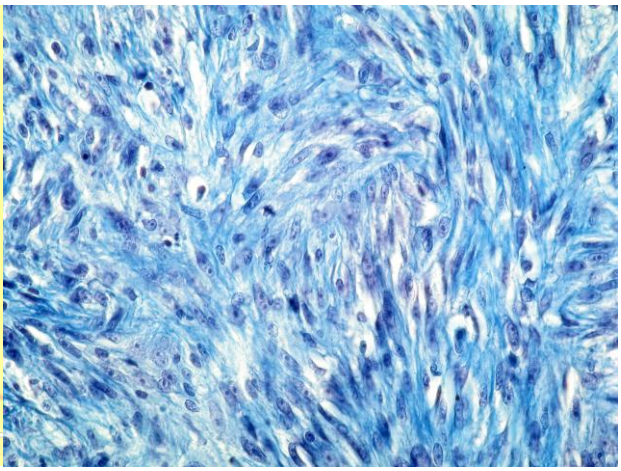

Figure 6. Fibrosarcoma. The neoplastic cells are arranged in intersecting cords. HEA stain. x40

The neoplastic cells are arranged in intersecting cords. The nuclei with marked anisokaryosis are located in a richly collagenic stroma (Fig. 6).

Malignant fibrous histiocytoma is one of the most aggressive MMT in dogs. Cellular pleomorphism, numerous multinucleated cells are usually seen in histopathological sections. The neoplastic cells are included in a richly collagenic mass, arranged in intersecting bundles and wirls (Fig. 7)

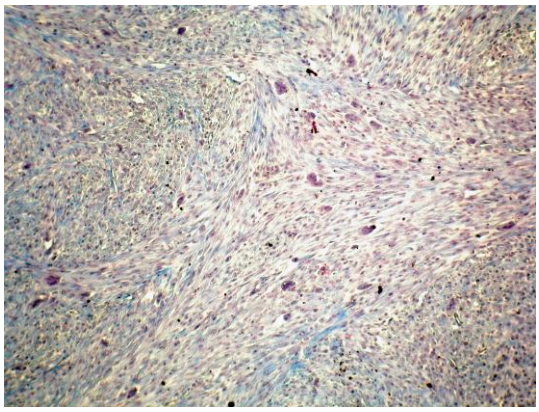

Figure 7. Malignant fibrous histiocytoma. Intersecting bundles of neoplastic cells and numerous multinucleated cells. HEA stain, x10

CONCLUSIONS

MMT represented 25.7% of the cases of cutaneous lesions presented for diagnostic pathology between 2007 and 2011.
The highest and the lowest incidence was in 2011 (26%) and in 2009 (14.7%), respectively.
There was no sex predilection. Out of the 325 dogs with MMT, 165 were males and 160 were females.
The mixed-breed and the cross-breed were overrepresented. MMT was diagnosed in Boxer, German Shepherd and Rottweiler, as well.
The mean age increased constantly, from 8 years in 2007, 9 years in 2011. The age range (1 month – 16 years) remained constant.
MMT developed mainly on the trunk (40% - 50%) and limbs (28% - 43%). The fewest lesions appeared on the neck (2% - 9%).
MMT is mainly a solitary lesion, but cases of multicentric location were noted.
Cytology by FNA or SEs was the main examination method.
MCT (29% - 48%), and hemangiopericytoma (18% - 35%) had the highest incidence of all the diagnosed MMTs.

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