OVERVIEW OF THE EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS OF THE CUTANEOUS MALIGNANT EPITHELIAL TUMORS IN DOG

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Abstract

The malignant epithelial tumors (MET) are frequent and very important in the pathology of the dog's skin. The aim of the present study was to evaluate these tumors, both epidemiologically and morphologically. During 2007-2011, a total of 3643 dogs have been specifically examined; 224 of them had MET. The specimens were obtained by fine needle aspiration (60%) and surgical biopsy (40%). During these five years, a total of 3643 dogs have been specifically examined and 1262 (34.5%) of them had cutaneous lesions, and 224 (17.7%) dogs were diagnosed with MET. The incidence of the cutaneous MET increased with a constant rate, from 12% in 2007, to 23% in 2011. No predilection of breed was observed, but the majority of the dogs were medium and large breeds. 57% were males and 43% were females. The median age of the affected dogs was 9 years. The neoplasms were located on the trunk (34%), head (32.5%), limbs (30.5), neck (3%). The attempt to correlate the incidence of the tumors with the season concluded that the majority of the diagnoses were established during spring (30.5%) with the fewest, during summer (12%). Cytological examination was the single method for investigating 47% of the surgical samples. Sole histological examination was used for 17% of the surgical samples and 36% of the cases were diagnosed by both methods of investigation. The most frequent MET were squamous cells carcinoma, (31%), tumors with adnexal differentiation - malignant trichoepithelioma, malignant pilomatricoma (24%) and basal cell carcinoma (21.5%), but rarely were diagnosed: sebaceous carcinoma, apocrine carcinoma, and eccrine carcinoma. A constant increase of the incidence of cutaneous MET was observed in dog and the importance of cytological and histological examination was also demonstrated.

Key words: *cutaneous, dog, epithelial, malignat, tumor*

MATERIALS AND METHODS

The study was conducted between January 2007-December 2011 in the Department of Pathological Anatomy, Faculty of Veterinary Medicine Bucharest. From a total of 3643 dogs specifically examined, 1262 were diagnosed with cutaneous lesions. Of those 1262 dogs with skin lesions, 224 were diagnosed with MET, this representing the cases included in the study. Was followed MET incidence, epidemiological aspects (breed, age, gender, location) and morphology and the importance of anatomopathological,

cytological and histological exams in the diagnosis of cutaneous lesions in dogs. For the cytological exam were performed fine needle aspiration (FNA) or imprinting of the operatory piece (OP). The smears performed for cytological exam were stained with May-Grunwald Giemsa (M-G G) or quick Giemsa. The histological exam assumed the harvesting of tissue fragments, they were processed through the classical histological method, with the inclusion in paraffin, sectioned at 4-6 microns and stained with Masson trichrome method and HE.

RESULTS AND DISCUSSION

Between January 2007-December 2011 in the Department of Pathological Anatomy, Faculty of Veterinary Medicine Bucharest 3643dogs were specifically examined. Of whom, 1262 (34.5%) were diagnosed with cutaneous lesions, and among them 224 have been diagnosed with MET, which represents 17.7% of all diagnosed skin lesions.

In the year 2007, 500 dogs were examined, of which 172 (34.4%) had presented skin lesions. Of these 39 (22.7%) were diagnosed with MET.

Table 1.

Presentation of diagnosed cases in 2007 with MET

MONTH	**CASES**	**SEX F/M**	**AGE years**	**LOCALIZATION**				**RECOLT-ATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	1	1M	5	0	0	1	0	1	0	0	0	0
Jan.	9	2F/7M	10	4	0	3	2	7	2	1	1	
Feb.	4	3F/1M	9	1	1	0	2	1	3	2	1	0
	14/36%											
March	6	2F/4M	11	1	0	1	5	1	5	0	3	2
April	5	2F/3M	8	2	0	2	2	2	3	0	0	3
May	3	1F/2M	11	1	0	0	2	2	1	0	0	1
	14/36%											
June	2	1F/1M	7	1	1	0	0	2	0	0	0	0
July	3	2F/1M	9	0	0	2	1	3	0	0	0	0
Aug.	1	1F	7	1	0	0	0	1	0	0	0	0
	6/15%											
Sept.	2	2F	11	0	0	1	1	2	0		0	0
Oct.	2	1F/1M	12	1	0	1	0	2	0	0	0	0
Nov.	1	1F	6	0	0	0	1	1	0	1	0	0
	5/13%											
Total	**39/100%**	**18F/21M**	**9**	**12**	**2**	**11**	**16**	**25**	**14**	**3**	**5**	**6**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP = operatory pieces, C+H=cytologic and histologic.

In the year 2008 has been recorded a total of 735 cases, 47% more than in 2007. Among these 277 (37.7%) presented skin lesions and 54 (19.5%) were diagnosed with MET.

Table 2.

Presentation of diagnosed cases in 2008with MET

MONTH	**CASES**	**SEX F/M**	**AGE years**	**LOCALIZATION**				**RECOLTATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	2	1F/1M	10	1	0	1	0	2	0	0	0	0
Jan.	4	1F/3M	9	0	2	1	1	1	3	1	0	2
Feb.	5	1F/4M	11	1	1	2	1	3	2	1	0	1
	11/20%											
March	5	2F/3M	10	0	1	3	1	5	0	0	0	0
April	5	5M	9	1	0	3	1	2	3	1	2	0
May	4	4M	7	1	1	0	2	3	1	0	1	0
	14/26%											
June	9	4F/5M	9	3	0	2	5	5	4	2	1	1
July	3	1F/1M	9	0	0	1	2	2	1	0	1	0
August	3	2F/1M	8	1	1	1	0	2	1	0	0	1
	15/28%											
Sept.	5	1F/4M	8	3	1	1	0	2	3	0	3	0
Oct.	4	4F	12	2	0	2	0	2	2	0	0	2
Nov.	5	3F/2M	9	2	1	1	1	3	2	0	1	1
	14/26%											
Total	**54/100%**	**20F/34M**	**9**	**15**	**8**	**18**	**14**	**32**	**22**	**5**	**9**	**8**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

In the year 2009, were examined 700 canine, 218 (31%) of them had skin lesions, of these 26, respectively 12% were diagnosed with MET.

Table 3

Presentation of diagnosed cases in 2009 with MET

MONTH	CASES	SEX F/M	AGE years	LOCALIZATION				RECOLTATION TYPE		DIAGNOSTIC TYPE OP		
				H	N	T	L	FNA	OP	C	H	C+H
Dec.	2	1F/1M	11	0	0	2	0	2	0	1	0	1
Jan.	4	1F/3M	9	2	0	0	2	3	1	2	1	1
Feb.	1	1F	11	1	1	0	0	1	0	0	0	1
	7/27%											
March	4	1F/3M	5	1	1	1	1	3	1	2	1	1
April	3	2F/1M	8	0	0	0	3	1	2	3	0	0
MaY	4	3F/1M	7	0	0	3	1	4	0	3	0	1
	11/42%											
June	1	1M	10	0	0	1	0	1	0	1	0	0
July	1	1 M	12	0	0	1	0	1	0	1	0	0
August	1	1 M	12	1	0	0	0	1	0	0	0	1
	3/12%											
Sept.	1	1 M	9	0	0	1	0	1	0	1	0	0
Oct.	2	2 M	8	1	0	1	0	2	0	1	0	1
Nov.	2	1F/1M	1	0	0	2	0	2	0	1	0	1
	5/19%											
Total	**26/100%**	**10F/16M**	**9**	**6**	**2**	**12**	**7**	**22**	**4**	**16**	**2**	**8**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

In 2010 has been registered an increase in the number of cases, compared to the previous years, respectively 807. Of those 807 dogs examined, 236 (29.5%) were diagnosed with cutaneous lesions, of whom 35, respectively 14.8% with MET.

Table 4

Presentation of diagnosed cases in 2010 with MET

MONTH	CASES	SEX F/M	AGE years	LOCALIZATION				RECOLTATION TYPE		DIAGNOSTIC TYPE OP		
				H	N	T	L	FNA	OP	C	H	C+H
Dec.	2	1F/1M	11	1	0	1	1	1	1	0	0	1
Jan.	1	1M	3	0	0	0	1	0	1	0	0	1
Feb.	4	2F/2M	11	1	1	1	1	1	3	2	0	1
	7/20%											

March	6	4F/2M	10	3	1	3	0	5	2	0	0	2
April	5	3F/2M	10	1	0	0	4	1	4	2	0	2
May	1	1F	11	0	0	1	0	1	0	0	0	0
	12/34%											
June	4	1F/3M	9	3	0	0	1	2	2	1	1	0
July	2	2F	9	1	0	1	0	2	0	0	0	0
August	1	1M	8	0	0	1	0	0	1	0	0	1
	7/20%											
Sept.	2	1F/1M	7	1	0	1	0	0	2	0	2	0
Oct.	3	1F/2M	11	2	0	1	0	2	1	0	0	1
Nov.	4	4M	8	2	1	0	1	0	3	1	0	2
	9/26%											
Total.	**35/100%**	**16F/19M**	**9**	**15**	**3**	**10**	**9**	**15**	**20**	**6**	**3**	**11**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C + H = cytologic and histologic.

In 2011 the number of examined cases was 901 dogs. Among those, 359 (39.8%) were diagnosed with cutaneous lesions, of which 70 or 19.5% presented TEM.

Table 5

Presentation of diagnosed cases in 2011 with MET

MONTH	**CASES**	**SEX F/M**	**AGE** years	**LOCALIZATION**				**RECOLTATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	9	6F/3M	9	5	0	2	2	3	6	2	1	3
Jan.	3	3F	8	0	1	1	1	3	0	0	0	0
Feb.	3	2F/1M	11	2	0	1	0	2	1	0	0	1
	15/21%											
March	7	3F/4M	11	5	0	0	2	5	2	0	0	2
April	7	3F/4M	10	4	0	2	1	7	0	0	0	0
May	3	1F/2M	11	0	0	1	2	2	1	0	0	1
	17/25%											
June	4	4M	9	1	0	1	2	2	2	0	1	1
July	7	5F/2M	11	1	0	3	3	5	2	0	0	2
August	4	1F/3M	12	0	0	3	1	2	2	0	0	2
	15/21%											
Sept.	9	3F/6M	10	3	0	4	4	6	3	1	1	1
Oct.	7	3F/4M	8	1	0	4	3	2	5	0	3	2
Nov.	7	3F/4M	9	3	0	3	2	4	3	0	1	2
	23/33%											
Total	**70/100%**	**33F/37M**	**9**	**25**	**1**	**25**	**23**	**43**	**27**	**3**	**7**	**17**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

According to the data presented, it can be observed that in 2011 was registered the highest number of diagnosed cases with skin lesions (n = 359),of which 70 presented MET and considering that the thermal comfort in the summer of 2011 was exceeded by high temperatures, over 35°C for long periods of time, can be suspected that the thermal factor, solar radiation in some cases, have been implicated as predisposing factors in triggering or activation MET. Although there is no one certain breed predisposition, breeds like Rottweiller, Cocker, German Shepherd, Caniche, but also their crossbreeds and common breeds presented a higher incidence of malignant epithelial tumors.

In the gender distribution was noted that in all five years studied, from 2007-2011, the most affected were males, with a rate of 54% in 2007, 63% in 2008, 61.5% in 2009, 54% in 2010, 53% in 2011, compared with females. In 2008 and 2009 we can notice a higher distance of males compared with females, on the incidence of malignant skin tumors, at the same time remarking that in the years 2007, 2010 and 2011, the gender differences are very low. Although the specialized literature indicates that the males are more likely to develop skin lesions, in the our studied cases it is shown that the gender differences are small or insignificant. Regarding to the location of malignant skin tumors, it can be seen that there are constant elements, but also fluctuates from year to year. Thus, the localization of the head and torso was maintained at a high level in 3 out of the 5 years studied, respectively in 2008, 2010 and 2011, except in 2009 when the first places were occupied by the locations on the torso and limbs, and also, except in 2007, when the first place was occupied by the limb localization, the only year in which the limb localization was paramount. Regarding to the location of malignant skin tumors, it can be seen that there are constant elements, but also fluctuates from year to year. Thus, the localization of the head and torso was maintained at a high level in 3 out of the 5 years studied, respectively in 2008, 2010 and 2011, except in 2009 when the first places were occupied by the locations on the torso and limbs, and also, except in 2007, when the first place was occupiedby the limb localization, the only year in which the limb localization was paramount.

In 2011, the head and torso localizations are maintained at an equal level, recording a maximum of incidence and closely followed by limb localization.
In 2007 limb localizations were the most numerous, while in 2008 and 2009 the torso localizations to sum up most of the cases, as well as head localization in 2010.Neck localization showed the lowest incidence, and in 2011 from a total of 70 cases, only in one case the lesions were located on the neck.
Fine needle aspiration and excisional biopsy represents the main methods of sampling intended for cytological and histological diagnosis.In general, in diagnosed cases with malignant epithelial skin tumors fine needle aspiration was the most frequently used, reaching a peak in 2011, just in 2010 the main sampling method was the excisional biopsy, and in 2008 have been equally used both sampling methods.Fine needle aspiration is an easily performed method, fast and cheap, allowing a rapid diagnosis (several hours) which makes it to be recommended by clinicians and preferred by owners.The high percentage recorded in 2011 it may be correlated with the economic crisis situation faced by our country, so that the owners preferred this method from economically reasons.It is true that the fine needle aspiration and cytological examination have their limits and some error margin, compared to operatory pieces and histological examination, whose relevance is higher.

Table 6

Collecting method of the used samples

Year	Cytological	Histological	Cytological& Histological
2007	11	5	23
2008	23	16	15
2009	16	2	8
2010	18	4	13
2011	38	10	22
	106	**37**	**79**

Collected samples through the fine needle aspiration puncture were examined and diagnosed only cytologically, while the excisional biopsy samples were examined and diagnosed or cytologically, or histologically, or also cytologically and histologically.As shown in chart 9 and the following table, the cytological examination has experienced significant increase in 2011, when 38 cases, respectively 54% were diagnosed through this method, compared to 2007 when many of the clinicians, but also the owners

preferred to perform both cytological and histological exams. The histological examination was preferred in 2008, following in frequency the cytological examination.

The lesional range encountered in the casuistry under study is presented in the following table:

Table 7

Anatomopathological examination results

Year	Cases	SCC	BCC	BSC	MTE	MP	AGC	EGC	SGC	MGC	CGC	Other MET
2007	39	10	13	0	8	1	2	2	0	0	0	3
2008	54	25	8	0	9	0	4	1	2	0	1	4
2009	26	9	4	0	4	6	0	1	0	0	0	2
2010	35	10	6	0	10	1	2	1	4	0	0	2
2011	70	15	17	2	16	2	1	0	3	1	1	12

SCC=squamous cell carcinoma, BCC=basal cell carcinoma,
BSC=basosquamous carcinoma, MTE=malignant trichoepithelioma,
MP=malignant pilomatrixoma, AGC=apocrine gland carcinoma,
EGC=eccrine gland carcinoma,SGC=sebaceous gland carcinoma,
MGC=Meibomian gland carcinoma, CGC=ceruminous gland carcinoma,
MET=malignant epithelial tumors.

According to presented data, is shown that the most diagnosed malignant epithelial tumor throughout the entire study period was the squamous cell carcinoma, followed by basal cell carcinoma.
Cytological diagnosis of squamous cell carcinoma has a high degree of relevance, especially in poorly differentiated tumors. Depending on the degree of differentiation, cellular atypia may be discrete in the case of well-differentiated carcinoma (Fig. 1) or severe in the poorly differentiated case (Fig. 2). Tumoral cells can be isolated or clusters in which case the intercellular desmosomal connections are often obvious.
The nuclei appear small and hyperchrome in well-differentiated carcinoma (Fig. 1) and large, with obvious anisokaryosis and prominent nucleoli in the poorly differentiated (Fig. 2). In the poorly differentiated type, in the cytoplasm can appear vacuolisation, located mainly perinuclear (Fig. 2). Constantly in squamous cell carcinoma, inflammatory cells are present, especially neutrophils, the keratin is an induced element of the inflammatory process (4, 5, 7).
Histological aspects have a high degree of specificity, which allows to establish easily the diagnosis. The cords and islands of epithelial cells with

varying degrees of squamous differentiation from the epidermis penetrate the dermis, and the keratin appears as oxyphil clusters, concentric, known as "keratosic pearls" (Fig. 3 and 4). In the poorly differentiated squamous cell carcinoma, the keratosic pearls may be missing, in the cytoplasm of the tumoral cells can be seen oxyphil tonofilaments of keratin (Fig. 5).

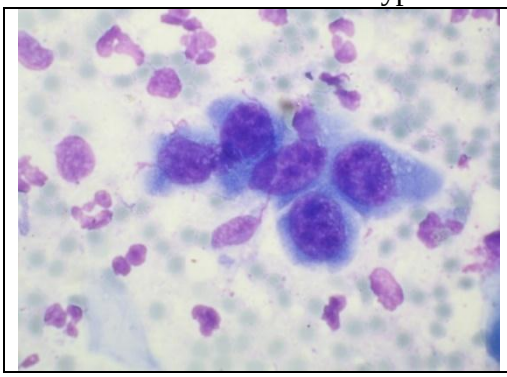

Fig.1.Poorly differentiated squamous cell carcinoma. Tumoral cells with severe anisokaryosis and obvious nucleoli. M-G G stain, 100x

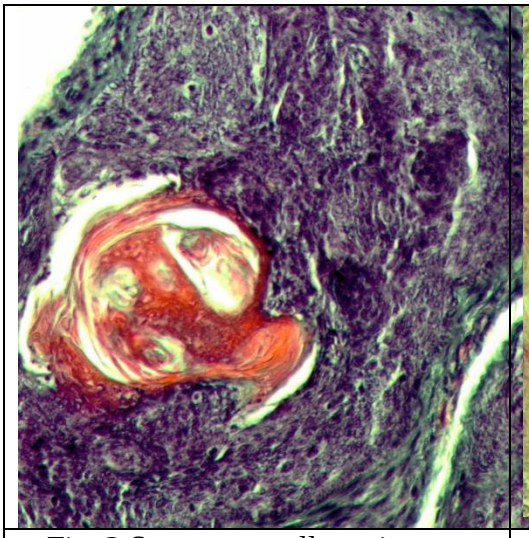

Fig. 2.Squamous cell carcinoma. Squamous cell proliferation and "keratosic pearls" inserted deep into dermis. Masson trichrome stain, 20x.

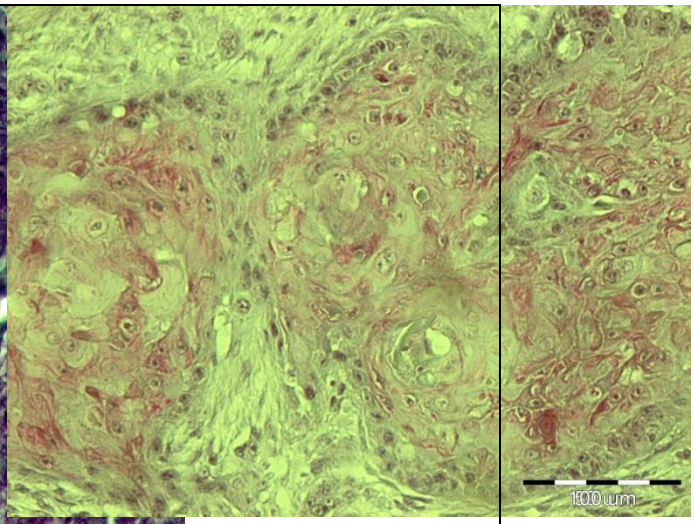

Fig. 3. Poorly differentiated squamous cell carcinoma. Anaplastic cells with intracytoplasmic keratine tonofilaments. Masson trichrome stain, 20x.

Basal cell carcinoma is an epithelial tumor with low degree of malignancy which can be differentiate either from the epidermis, or from the hair follicle epithelial structures.
In cytologically terms is characterized by cohesive sheet or ribbon of palisading epithelial cells, uniformly in shape and size, with N:C ratio generally 1:1, and only rarely dysplastic or with a reduced anaplastic grade (Fig. 6). Sometimes, the cells presents sebaceous differentiation, which makes difficult the differential diagnosis of this type of neoplasm and sebaceous gland tumors (2, 8). In these cases the histological examination allows to establish the correct diagnosis.

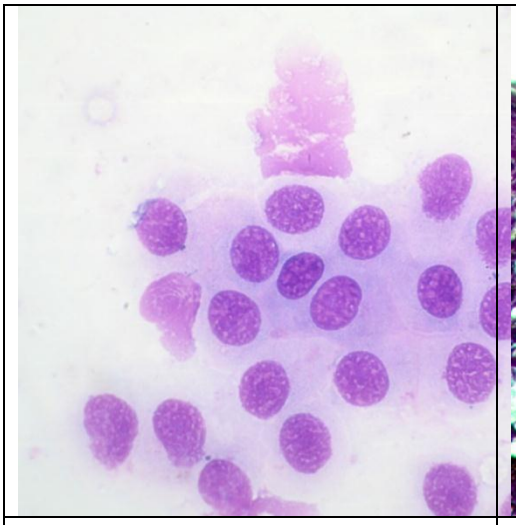

Fig. 6.Basal cell tumor. Epithelial cells of basal type, dysplastic and with a low anaplastic degree. M-G G stain, 100x

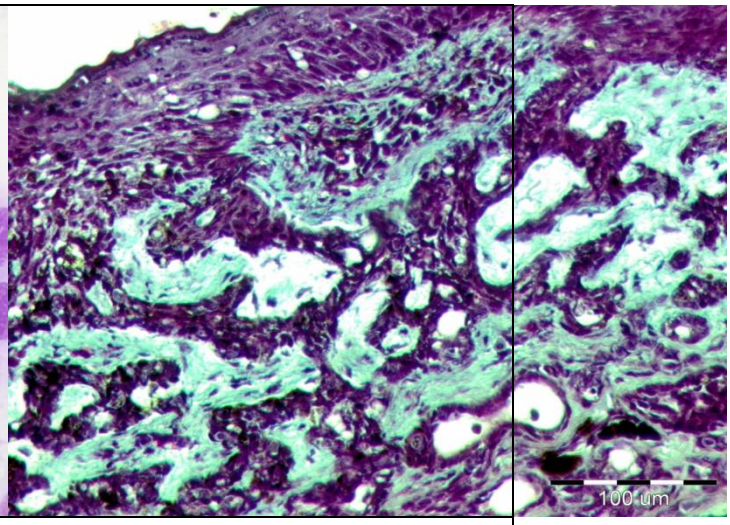

Fig. 7.Basal cell carcinoma - infiltrative type. Cords of epidermal epithelial cells infiltrated into the dermis. Masson trichrome stain, 20x.

Histological aspects encountered in basal cell carcinoma can be of two types: infiltrative or with clear cells (1, 4, 7). Histological examination had been established the diagnosis of basal cell carcinoma infiltrating type. Can be observed cords and tapes of basal epithelial cell type, with hyperchrome nuclei and reduced cytoplasm, which start from the epidermis to the dermis, inserting among its structures (Fig. 7).
Sebaceous gland carcinoma is a relatively rare tumor in dogs (2, 3). In cytologically terms is characterized by the presence of sebaceous cells with varying degrees of pleomorphism, with intracytoplasmic optical empty

vacuoles. Cytologically is difficult to differentiate the different types of sebaceous gland tumors (adenoma, epithelioma, carcinoma), and also differentiation of the sebaceous gland tumors of the basal cell tumors with sebaceous differentiation (2, 6, 8).
The histological examination allows to differentiate these lesions. Sebaceous gland carcinoma is characterized by the presence of sebaceous cells with moderate pleomorphism, with varying degrees of lipid charging of the tumoral cells (Fig. 8). The nuclei are large, with obvious nucleolus, sometimes atypical mitoses are detected.

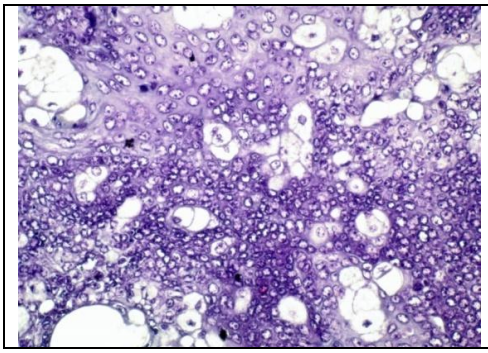

Fig. 8. Sebaceous gland carcinoma. Sebaceous epithelial cell type with moderate pleomorphism, with varying degrees of intracytoplasmic lipid charging, with large nuclei, with evident nucleolus. Masson trichrome stain, x40

Apocrine gland carcinoma develops from the epithelial secretory cells of apocrine glands. Are relatively common in dogs, are considered aggressive tumors with high metastatic potential. In histologically terms it may be encountered forms: solid, tubular and cystic. In our case has been detected the solid type, the most common type of apocrine gland carcinoma.
This type is characterized by proliferation of the epithelial cells, in the form of islands, separated by connective tissue. The nuclei are round or oval, pleomorphic, with evident nucleoli (Fig. 9).

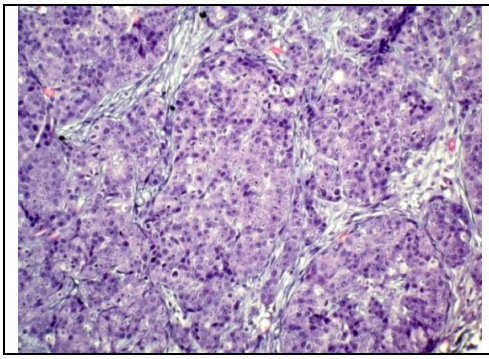

Fig. 9. Apocrine gland carcinoma.Proliferation of epithelial cells, with round or oval nuclei, pleomorphism with evident nucleoli, separated by connective tissue. Masson trichrome stain, 40x

CONCLUSIONS

Of the 1262 diagnosed dogs with cutaneous lesions, 224 (17.7%) presented malignant epithelial tumors.
The lowest incidence of malignant epithelial tumors was registered in 2009 - 12%, and the highest in 2007 - 22.7%.
The most affected breeds were Cocker, German Shepherd, Caniche, Rottweiler, but especially their crossbreeds.
The average age of diagnosed animals with MET was 9 years, maintaining constant throughout the 5-year study considered.
Males were more affected (56.69%) compared to females (43.30%).
The most common sites were on the torso, head and limbs.
The most utilized method was the cytopathology diagnosis through the fine needle aspiration puncture.
The most common diagnosed malignant epithelial tumors were squamocellular carcinoma (31.25%), basal cell carcinoma (21.42%), malignant trichoepithelioma (19.64%).

REFERENCES

Baba Alexandru Ioan (2002) – Comparative oncology, Ed. Academiei Române, Bucharest.
Baker Rebecca, Lumsden John H. (1999) – Color atlas of citology of the dog and cat, Ed. Mosby.
Goldschimdt M. H., Dunstan R. W., Stannard A. A., Tscharner, Walder E. J., Yager J. A. (1998) – Histological classification of epithelial and melanocytic tumors of the skin of dometic animals, Second series, vol. III, Ed. Armed Forces Institute of Pathologs.
Gross T.L. , Irhe P.J., Walder E. J., Affolter V. A. (2005) – Skin diseases of the dog and cat. Clinical and histopathological diagnosis. Second edition, Ed. London Blackwell.
Jubb K.V.F., Kennedy Peter C., Palmer Nigel (1993) – Pathology of domestic animals, vol. I, Academic Press, New York.
Manolescu Nicolae, Balint Emilia (2009) – Atlas of canine and feline oncocytomorphology. Ed. Curtea Veche, Bucharest.
Moulton E. Jack (1990) – Tumors in domestic animals, Third edition, revised and expanded, Ed. University of California Press, Berkeley, L. A. London.
Raskin Ross E., Meyer Danny J. (2010) – Canine and feline cytology, a color atlas and interpretation guide, Ed. Saunders Elsenier.