Cytomorphological characterization of transmissible canine venereal tumor

Caracterização citomorfológica do tumor venéreo transmissível canino

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Summary: Canine transmissible venereal tumor (TVT) has been the object of numerous investigations since 1876, when Novinsky reported the first successful experimental transplant of a tumor using this neoplasia. Cytology must be the method of choice for the diagnosis of suspected TVT, since it is a simple technique, minimally invasive and painless and, furthermore, produces much less distortion of cell morphology than biopsy samples fixed in formalin. Mohanty and Rajya (1977) described that TVT cells *in vitro* presented two morphologically distinct types. The classification of TVT based on cell morphology has been adopted by the Veterinary Pathology Service of FMVZ-UNESP, Botucatu, Brazil, since 1994. However, there still is no study consolidating the utilization of this classification in TVT. As the morphology and function are two intimately related characteristics and morphological differences must always be investigated such that adequate treatment would be employed and, as the existence of two TVT subtypes has already been affirmed, this article aims to standardize a new international TVT nomenclature. To this end, one hundred and thirty-two dogs were utilized - without restriction as to sex, age or breed - with cytological and clinical diagnoses of transmissible venereal tumor (TVT) attended to at the Veterinary Hospital of FMVZ-UNESP, Botucatu, Brazil. The most frequent localization was genital, followed by nasal; 25.2% of patients presented metastases. These occurred most frequently on the skin (31% of metastases), followed by mammary localization in females and lymph nodes in males. Samples were collected from 158 tumors for cytological evaluation by optical microscope. The masses were evaluated according to localization, either genital or extragenital, and biological behavior, in primary or non-primary ones (metastatic or recurrent). The cytological evaluation included classification according to the predominant morphological pattern: lymphocytic (18.36%), plasmacytic (52.53%) or mixed (29.11%). The extragenital and non-primary samples presented predominantly a plasmacytic pattern. The results permited the conclusion that the morphological differences in transmissible-venereal-tumor cells are related to more aggressive biological behavior in the plasmacytic pattern, by its capacity to develop in extragenital locations and metastasize.

Resumo: O tumor venéreo transmissível (TVT) canino tem sido objeto de numerosas investigações desde 1876, quando Novinsky relatou o sucesso do primeiro transplante experimental de um tumor usando esta neoplasia. A citologia deve ser o método de escolha para o diagnóstico de suspeitas de TVT, pois é uma técnica simples, minimamente invasiva e indolor e, além disso, produz muito menos distorção da morfologia celular do que as amostras de biópsia fixadas por formalina. Mohanty e Rajya (1977) descreveram que células de TVT *in vitro* apresentaram-se sob dois tipos morfologicamente distintos. A classificação do TVT baseada na morfologia celular é adotada pelo Serviço de Patologia Veterinária da FMVZ-UNESP, Botucatu, Brasil, desde 1994. Porém ainda não havia trabalho consolidando a utilização desta classificação no TVT. Como morfologia e função são duas características intimamente relacionadas e as diferenças morfológicas devem ser sempre investigadas de modo que um tratamento adequado seja empregado e como já se afirma existirem 2 subtipos de TVT, este artigo visa padronizar a nova nomenclatura do TVT em todo o mundo. Para tal, foram utilizados 132 cães sem restrição de sexo, idade ou raça, com diagnóstico clínico e citológico de tumor venéreo transmissível (TVT), atendidos no Hospital Veterinário da FMVZ-UNESP, Botucatu, Brasil. A localização mais frequente foi a genital, seguida pela nasal; 25,2% dos pacientes apresentavam metástases. Estas ocorreram mais frequentemente na pele (31% das metástases), seguida pela localização mamária, em fêmeas, e em linfonodos, nos machos. Foram colhidas amostras de 158 tumores para avaliação citológica por microscopia ótica. As massas foram avaliadas de acordo com a localização, em genitais ou extragenitais, e com o comportamento biológico, em primárias e não primárias (metastáticas ou recorrentes). A avaliação citológica incluiu a classificação de acordo com o padrão morfológico predominante, em linfocitóide (18,36%), plasmocitóide (52,53%) ou misto (29,11%). As amostras extragenitais e não primárias foram predominantemente do padrão plasmocitóide. Os resultados encontrados permitem concluir que as diferenças morfológicas nas células do tumor venéreo transmissível estão relacionadas a um comportamento biológico mais agressivo do padrão plasmocitóide, quanto à sua capacidade de se desenvolver em locais extragenitais e metastatizar.

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Introduction

Canine transmissible venereal tumor (TVT) has been the object of numerous investigations since 1876, when Novinsky reported the first successful experimental transplant of a tumor using this neoplasia (Cohen, 1978; Murgia *et al*., 2006). This tumor is transmitted

by implantation of viable tumor cells in mucous membrane, especially if there are abrasions or loss of integrity on the surface (Cohen, 1978; Rogers, 1997; Murgia *et al*., 2006).

Naturally occurring TVT generally develops in the external genitalia (Otomo *et al*., 1981; Rogers *et al*., 1998; Amaral *et al*., 2004). Despite this, TVT occurs in the nasal and / or oral cavity, skin and conjunctive mucosa, occurring together (Hamir, 1985; Varaschin *et al*., 2001; Amaral *et al*., 2004) or not (Bright *et al*., 1983) with genital TVT, probably as a consequence of social behaviors (Cohen, 1985; Amber e Adeyanju, 1986; Pérez *et al*., 1994; Ginel *et al*., 1995).

The primary ocular masses develop from conjunctiva, as reported by Boscos *et al*. (1998) and in nasal cases, may originate from another dog or as a result of auto-implanting starting from a genital mass (Miller *et al*., 1990; Pereira *et al*., 2000; Rodrigues *et al*., 2001). Batamuzi and Bittegeko (1991) reported one case of anal and genital TVT, assuming that the anal implant was possible due to the existence of a generalized cutaneous allergic process that could have produced perianal inoculation of neoplasic cells by the association of itching with injured skin.

The presence of metastases in naturally occurring TVT suggests an estimated rate between 1.5 and 6%, according to Ferreira *et al*. (2000), or from 0 to 17%, according to Rogers (1997) and MacEwen (2001). TVT can present metastases in multiple organs, including subcutaneous ones, or in the lung, anterior mediastinum, liver, spleen, kidneys and in superficial and deep lymph nodes of the thoracic and abdominal cavity (Park *et al*., 2006), as well as in the tongue, pharynx (Ndiritu *et al*., 1977), adenohypophysis (Manning and Martin, 1970) and even the brain (Kroger *et al*., 1991; Ferreira *et al*., 2000).

Independent from the malignant potential of this neoplasia, TVT is singular in its responsiveness to a variety of treatments (Rogers, 1997). Among the various modalities of treatment, chemotherapy is accepted as the most effective (Erünal-Maral *et al*., 2000). A therapy with vincristine sulfate as the only agent, in weekly applications, is the most effective protocol, with four to eight intravenous applications being necessary to obtain a cure (Erünal-Maral *et al*., 2000). In tumors resistant to vincristine, the drug of choice is doxorubicin (Rogers, 1997).

Cytology must be the method of choice for diagnosis of suspected TVT, since the technique is simple, cheap, minimally invasive, and painless and, furthermore, produces much less distortion of cell morphology than biopsy samples fixed in formalin (Daleck *et al*. 1987; Erünal-Maral *et al*., 2000; Bassani-Silva *et al*., 2003; Amaral *et al*., 2004). This exam has been used widely in diagnosis of TVT. Even in masses localized extragenitally (principally when there is necessity of imaging for diagnosis), and that do not include TVT as a differential diagnosis, the cytological exam was

shown to be efficient and conclusive in diagnosing this neoplasia (Amaral *et al*. 2004).

Cytological samples of TVT are generally multicellular and contain round or oval cells that vary between 14 and 30 μ in diameter, with well-delimited cytoplasmic borders. The nucleus, round or oval, is frequently eccentric, of variable size, with rough and granular chromatin and with one or two prominent nucleoli (Wellman, 1990). The nucleus:cytoplasm ratio is relatively high (Boscos *et al*., 1999). Until 2003, the literature on transmissible venereal tumor, of natural occurrence or transplanted experimentally, had not reported on different cell types, despite descriptions found supporting these differences, including: the absence of cytoplasmic vacuoles (Rogers, 1997) and the presence of larger and more ovoid cells in relation to the typical TVT cell morphology (Boscos *et al*., 1999). Varaschin *et al*. (2001) registered malignant TVTs presenting abundant cytoplasm. This morphology corresponds to plasmacytic TVT that, in studies accomplished by our research group, were shown to be more aggressive than the lymphocytic form (Amaral, 2005; Gaspar, 2005; Bassani-Silva, 2007). Many times the cellular aspect can vary between the primary tumor and the metastasis (Rogers, 1997; Boscos *et al*., 1999), or can be atypical in cases of old tumors (Boscos *et al*., 1999). Furthermore, Mohanty and Rajya (1977) described how TVT cells *in vitro* presented two morphologically distinct types: one, small cells with round to oval nucleus; and the other, large cells with hyperchromatic nucleus and acidophilic cytoplasm. In 2003, Bassani-Silva *et al*. had already reported the existence of varied degrees of aggressiveness, due to different cell lineages of TVT, with variable biological behavior. This existence of different TVT cell lineages was confirmed by Murgia *et al*. (2006) who, analyzing an amplified mitochondrial DNA fragment, observed differences in the sequence between the tumors and, therefore, that the ancestral clone of the tumor is divided into two subclasses, each of which is distributed among many countries.

Associated with modifications in morphology is the fact that old tumors do not respond well to chemotherapy (Boscos *et al*., 1999), which is more effective the earlier the diagnosis (Pérez *et al*., 1994). One hypothesis is the existence of different TVT cell lineages, characteristics that would influence their biological behavior, such as the ability to produce erythropoietin or to metastasize (Rogers *et al*., 1998).

The Veterinary Pathology Service of FMVZ-UNESP, Botucatu, Brazil, since 1994, has employed a TVT classification based on cell morphology (Bassani-Silva *et al*., 2003; Amaral *et al*., 2004). This adopted classification resulted from the hypothesis of the existence of different TVT cell lineages with respect to characteristics that influence its biological behavior, suggesting that this neoplasia presents various degrees of aggressiveness, since TVTs of

plasmacytic morphology presented higher frequency of nuclear abnormalities, associated with greater expression of glycoprotein-P and augmented resistance to antitumoral action of propolis with almost all cases of metastasis being of the plasmacytic type. In this context, it is concluded that this type is more aggressive, in other words, more malignant compared to those of lymphocytic or mixed morphologies (Amaral, 2005; Gaspar, 2005 Bassani-Silva, 2007).

Many authors utilize the classification of TVT into atypical and typical, although this classification does not appear adequate, since it is known that there are differences in the prevalence of a determinate cell type according to the region studied. In the southeastern region of Brazil the predominant TVT is plasmacytic (Amaral *et al.*, 2004) while the southern region of the country TVT of lymphocytic morphology predominates (non-published data).

Although the classification of TVT based on cell morphology has been employed by the Veterinary Pathology Service of FMVZ-UNESP, Botucatu, Brazil, since 1994, there is still no work consolidating the utilization of this TVT classification. As morphology and function are two intimately related characteristics (Wilkening *et al.*, 2003) and morphological differences must always be investigated in a manner to ensure that adequate treatment is employed, and as Murgia *et al.* (2006) have already affirmed the existence of two subclasses, the present article aims to standardize the utilization of a new nomenclature for TVT throughout the world.

Material and methods

The present study was approved by the Chamber of Ethics in Animal Experimentation of FMVZ-UNESP, *campus* at Botucatu, Brazil. In its development, 132 dogs were utilized without restriction as to sex, age or breed, with cytological and clinical diagnosis of transmissible venereal tumor, attended at the Veterinary Hospital of FMVZ-UNESP. The inclusion of patients was conditional in requiring free and informed consent of the dog owners.

Each tumoral mass was classified according to its localization (as *genital* or *extragenital*) and biological behavior (as *primary*, *metastatic* or *recurrent*), which were considered the experimental units.

In dogs with confirmed diagnosis of transmissible venereal tumor, samples were collected from 158 tumoral masses by aspirative cytology with a fine needle (CAAF), as described by Cowel and Tyler (1989), to make cytological preparations utilized for cytomorphological characterization. Two slides of each tumoral mass were left to dry in the open air, fixed in methanol and colored by the method of Giemsa.

A 24 3/4G caliber needle was utilized independent of

the size of the tumor to be aspirated. In cases in which visualization of the genital mass was not possible, as well as in cases of intranasal masses, a gynecological brush was used for collection of cells. In some cases tumoral fragments were loosened during clinical exam and were utilized to make cytological preparations by the impression technique.

Cytological preparations were analyzed under optical microscope to be selected for the tumors specific experimental groups, according to the characteristics of the predominant cell type, in the following manner:

o Group L (Lymphocytic): predominance of 60% or more of TVT cells with round morphology, scarce and finely granular cytoplasm, with the presence of vacuoles tracking the cell periphery, round nucleus with rough chromatin and the presence of one or two salient nucleoli (Figure 1A).

• Group P (Plasmacytic): predominance of 60% or more of TVT cells with ovoid morphology, more abundant cytoplasm (lower nucleus:cytoplasm ratio), with eccentrically located nucleus (Fig 1B).

• Group M (Mixed): mixed cellularity between lymphocytic and plasmacytic cell types, in which none surpassed 59% of the total.

The cytological preparations were evaluated initially with 10x objective for verification of cellularity of the sample, pattern of coloration and distribution of cells. Next observations were made at progressive increases amplification from of 250x to 400x to detail cell characteristics and to count the cells. After initial scanning, the areas with the best pattern of cellular distribution and coloration were analyzed at 400x, with a minimum of ten random non-superimposed fields counted on each slide to obtain classification of predominant cell type. Carefully detailed cytomorphological analysis was accomplished with the aid of a reticulated glass (grade of integration 100/25, with 25 mm^2 area), which delimited the cell-count field. At least 100 cells were counted from each sample; the cells selected were categorized according to morphology in lymphocytics or plasmacytics.

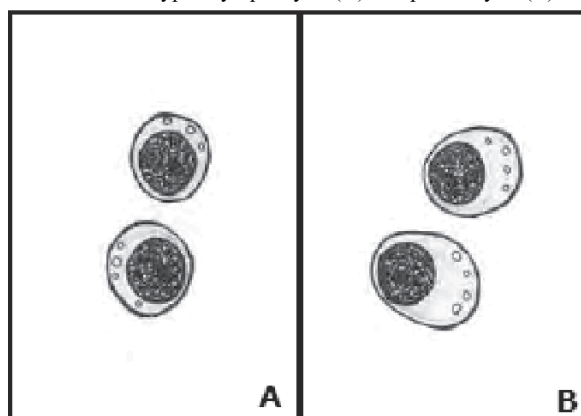Figure 1 - Cytomorphological scheme of transmissible canine venereal tumor types: lymphocytic (**A**) and plasmacytic (**B**)

Table 1 - Classification of neoplasic mass samples, based on localization and biological behavior

	CLASSIFICATION OF NEOPLASIC MASSES			TOTAL
	LOCALIZATION			
	Genital		Extragenital	
n (%)	120 (63.8%)		68 (36.2%)	188
	BIOLOGICAL BEHAVIOR			
	Primary	Metastatic	Recurrent	
n (%)	121 (64.4%)	55 (25.2%)	12 (6.4%)	188

Table 2 - Frequency of neoplasic masses of lymphocytic, mixed and plasmacytic groups, according to localization

LOCALIZATION	GENITAL		EXTRAGENITAL		TOTAL	
GROUP	n	%	n	%	n	%
LYMPHOCYTIC	26[a]	89.7	03	10.3	29	100
MIXED	34a[b]	73.9	12	26.1	46	100
PLASMACYTIC	49[b]	59.0	34	41.0	83	100
TOTAL	109	–	49	–	158	–

[a, b] Different letters represent significant difference among groups by the test of Goodman (p=0.05)

Data were analyzed at the Department of Biostatistics in the Institute of Biosciences of UNESP – Campus at Botucatu, Sao Paulo, Brazil.

For comparison between the morphology of TVT cells and the localization of the tumor (genital or extragenital) and biological behavior (primary or non-primary masses), association tests were utilized, based on the Chi Square statistic (Zar, 1996).

Results

Approximately two thirds of the tumors were primary and of genital localization (63.8% and 64.4%, respectively), with only 6.4% recurrent and 25.2% metastatic (Table 1).

The cytological preparations were analyzed and included in one of the experimental groups, defined as L, M or P, described previously. Lymphocytic cells tended to be smaller than plasmacytic ones. The plasmacytic pattern was the most frequent, with 52.53% of cases, followed by mixed (29.11%) and, finally, lymphocytic (18.36%). Figure 2 illustrates cytological sample characteristics of each group.

Frequency comparisons of tumors in the three groups (L, P, M) with localization and biological behavior are presented in Tables 2 and 3. These data were analyzed by statistical tests based on Chi Square (χ^2), with application of the Goodman test for comparison of multi-nominal proportions. Significant difference was detected among groups in comparison between localization genital and extragenital (Table 2, Figure 3) and between categories of biological behavior (Table 3). Of the tumors with genital localization, the lymphocytic group presented a concentration of 82.76%, while in the mixed group, this number was reduced to 76.09% and, in the plasmacytic group, reduced even more, down to 62.65%.

In relation to biological behavior, there also was significant difference between groups. To perform

Figure 2 - Cytological samples of transmissible venereal tumor of different cytomorphological types: A. lymphocytic pattern (predominance of round cells, scarce cytoplasm and high nucleus:cytoplasm ratio); B. plasmacytic pattern (predominance of ovoid cells, ample cytoplasm and eccentric nucleus); C. mixed pattern (presence of both morphological types without predominance of either). Giemsa, bar = 20μm
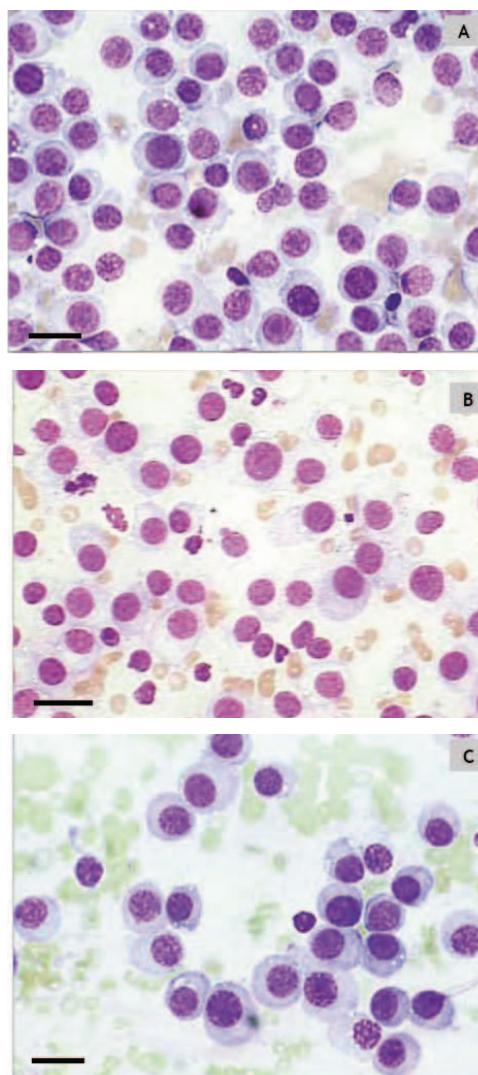
Table 3 - Frequency of neoplasic masses of lymphocytic, mixed and plasmacytic groups, between primary and non-primary tumors (metastatic or recurrent)

BIOLOGICAL BEHAVIOR	PRIMARY		NON-PRIMARY		TOTAL	
GROUP	n	%	n	%	n	%
LYMPHOCYTIC	26[a]	89.7	03	10.3	29	100
MIXED	34a[b]	73.9	12	26.1	46	100
PLASMACYTIC	49[b]	59.0	34	41.0	83	100
TOTAL	109	–	49	–	158	–

[a, b] Different letters represent significant difference among groups by the test of Goodman (p=0.006)

Table 4 - Frequency of lymphocytic and plasmacytic cells in cytological samples of transmissible venereal tumor according to localization and biological behavior of the tumor
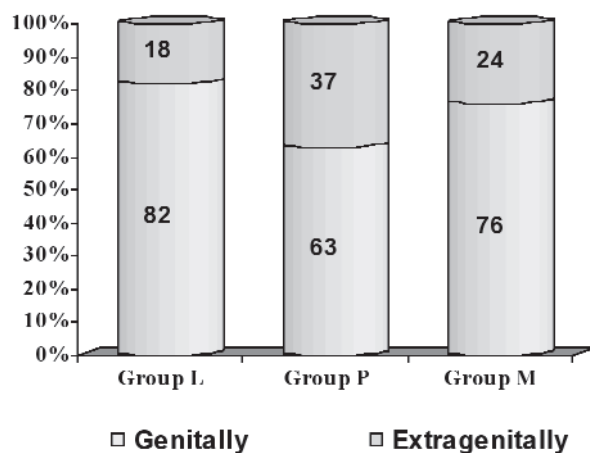
	LOCALIZATION							
	LYMPHOCYTIC CELLS				PLASMACYTIC CELLS			
	MEDIAN (%)	P_{25} (%)	P_{75} (%)	p	MEDIAN (%)	P_{25} (%)	P_{75} (%)	p
GENITAL	42[a]	32.25	55.00	0.05	59[a]	45.0	67.75	0.06
EXTRAGENITAL	38[b]	31.00	46.50		62[a]	53.5	69.00	
	BIOLOGICAL BEHAVIOR							
	LYMPHOCYTIC CELLS				PLASMACYTIC CELLS			
	MEDIAN (%)	P25 (%)	P75 (%)	p	MEDIAN (%)	P_{25} (%)	P_{75} (%)	p
PRIMARY	42[a]	33.75	57.25	0.005	58[a]	42.75	66.25	0.006
NON-PRIMARY	37[b]	30.75	42.25		63[b]	57.75	69.25	

[a, b] Different letters represent significant difference by the test of Mann-Whitney

statistical analysis, the metastatic and recurrent masses were grouped as non-primary masses to increase the homogeneity between categories. In the lymphocytic group only 10.3% were non-primary masses, a frequency that rose to 26.1% in tumors of the mixed cytomorphological group and to 41% in tumors of the plasmacytic group. Table 3 and Figure 4 present these data in detail.

Analysis of percentages of lymphocytic and plasmacytic cells, according to tumor localization and biological behavior, revealed significant differences – lymphocytic cells showed predominance in genital masses compared to extragenital ones, and of plasmacytic cells in non-primary tumors compared to primary ones. These observations are found in Table 4.

Discussion

Genital tumors were the most frequent, comprising 63.8% of the cases. The cases of nasal, ocular, anal and subcutaneous TVT were considered primary due to transmission by cellular implant. This casuistry is similar to that reported by Weir *et al.* (1978), Batamuzi and Bittegeko (1991), Ginel *et al.* (1995) and Rogers *et al.* (1998).

A total of 33 patients in the study (25%), presented dissemination of primary tumor, a value slightly higher than has been reported, varying between 0 and 17% in naturally occurring cases (Rogers, 1997; MacEwen, 2001).

The most frequent metastatic form was subcutaneous,

Figure 3 - Frequency of genitally and extragenitally localized tumors according to cytomorphological group. Distributions of lymphocytic and plasmacytic groups differed statistically

Figure 4 - Frequency of neoplasic masses of the lymphocytic, mixed and plasmacytic groups between primary and non-primary tumors (metastatic or recurrent)
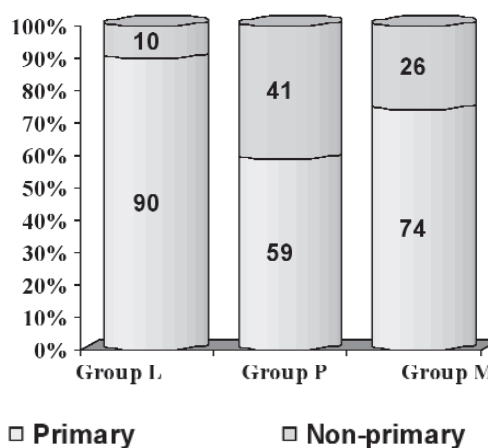
with 13 cases (31% of metastases). A similar finding was registered by Brandão *et al.* (2002), relating 127 cases of TVT, of which 22 were cutaneous masses. The cutaneous metastases are well-described in the literature consulted (Oduye *et al.*, 1973; Ndiritu *et al.*, 1977; Pandey *et al.*, 1989; Miller *et al.*, 1990; Kroger *et al.*, 1991; Ayyappan *et al.*, 1994; Guedes *et al.*, 1996; Boscos *et al.*, 1999). In males, neoplasic invasion was observed most frequently in inguinal lymph nodes and, in females, in mammary tissue – in these locations there was a significant difference between sexes. No reports of mammary transmissible venereal tumor were found in the literature, but metastasis in regional and distant lymph nodes is well-established (Oduye *et al.*, 1973; Ndiritu *et al.*, 1977; Yang, 1987; Ayyappan *et al.*, 1994; Rogers *et al.*, 1998; Das and Das, 2000). In cases evaluated in the present study metastases were verified in lymph nodes in only two females (inguinal in one, internal iliac in the other), while nine males presented metastases in inguinal lymph nodes. This distribution appears to be related to lymphatic drainage of the external genitalia, done exclusively to the inguinal lymph nodes in males, and to inguinal lymph nodes and iliac internal ones in females. The linking of distribution of metastases with differences in lymphatic draining in the two sexes was proposed by Oduye *et al.* (1973). As diagnostic exams by abdominal imaging were not performed in all cases, it is possible that a greater number of females would present metastases in the internal iliac lymph node. The neoplasic cells, in some manner, were able to bypass the inguinal lymph nodes and fix in the mammary tissue.

Approximately two thirds of sampled tumors were of genital and primary localization (63.8% and 64.4%, respectively), with only 6.4% recurrent and 25% metastatic, according to TVT behavior, following MacEwen (2001).

The use of cytological exam for diagnosis of neoplasias of round cells was recommended by Duncan and Prasse in 1979, when these authors considered it more advantageous than the histopathological exam. Since then, the cytological exam has been recommended as essential to diagnosis (Amaral *et al.*, 2004; Meinkoth and Cowell, 2002) and monitoring of treatment (Batamuzi and Kessy, 1993; Erünal-Maral *et al.*, 2000) of transmissible venereal tumor, being widely used. Brandão *et al.* (2002) reported that out of 127 TVT cases, 94.6% were diagnosed by cytological exam.

The most frequent cytomorphological pattern was plasmacytic, comprising 52.53% of cases, followed by mixed (29.11%) and, finally, lymphocytic (18.36%). A statistically significant difference was detected between the lymphocytic and plasmacytic groups in the comparison between localization (genital and extragenital) and between categories of biological behavior. The lymphocytic group presented a incidence of 82.76% of genitally located tumors, while in the plasmacytic group, this number was reduced to 62.65%; only 10.3% of the lymphocytic group presented non-primary masses, versus 41% of plasmacytic group tumors. In a descriptive study completed by our research group, out of 576 cases of TVT there also was predominance of plasmacytic TVT type in genital and extragenital masses (Amaral *et al.*, 2004). Mohanty and Rajya (1977) described the occurrence of two distinct morphological types of TVT cells in culture *in vitro*, one cell line composed of small cells with nucleus round to oval and vesicular, and the other one showing large cells with a large hyperchromatic nucleus. Boscos *et al.* (1999) reported the presence of ovoid cells in cutaneous transmissible venereal tumor – this description is highly similar to that of the plasmacytic pattern, which in our findings was significantly superior / greater, in the extragenital masses, to the lymphocytic pattern, which is closer to the morphology usually described for neoplasia.

As the cutoff point for the number of cells for inclusion in groups was relatively arbitrary, in addition to the comparison among cytomorphological groups, a statistical analysis was performed on percentages of lymphocytics and plasmacytics cells versus tumor localization and biological behavior. This analysis revealed significant differences – there is predominance of lymphocytics cells in genital masses (median: 42 cells) when compared to extragenital ones (median: 38), and in the primary masses (median: 42 cells) compared to non-primary (median: 37 cells). Plasmacytics cells presented a median significantly higher in non-primary tumors (median: 63) compared to primary ones (median: 58 cells). These findings corroborate the previous analysis, and lead to the belief that the limit established for the division of groups was adequate.

These comparisons indicate the association between the plasmacytic phenotype with extragenital localization, with non-primary masses (metastases or recurrences) and with the capacity to develop metastases.

Of all the masses analyzed, only two of extragenital localization, metastatic, were encompassed in the lymphocytic group and only two cases of lymphocytic primary morphology developed metastases – these were from one of the other two groups (plasmacytic and mixed). Confirming our observation, Rogers (1997) emphasized that the primary and metastatic masses cannot have identical cytological appearance. Ferreira *et al.* and Pereira *et al.*, in 2000, described cases of intraocular TVT in which the histological pattern was so "uncommon" that immunohistochemical studies were necessary to confirm the diagnosis. Even though the description was generic, this uncommon pattern may be a plasmacytic-type manifestation. Rogers *et al.* (1998) supposed the existence of different lineages of transmissible venereal tumor in relation to characteristics that influence biological behavior, such

as ability to metastasize. Finally, corroborating our findings is the study of Murgia *et al.* (2006), which confirms the existence of two subclasses of TVT.

Conclusions

Based on the findings obtained during the experiment, we can conclude that: the cytomorphological characteristics of the different cell patterns of transmissible venereal tumor permit a distinction between two cell types, lymphocytic and plasmacytic, so that TVT can be classified into plasmacytic, lymphocytic and mixed. The findings on prevalence of cytomorphological types according to localization and biological behavior and on the cell population of tumors with and without metastases point to a greater ability of plasmacytic cells to develop in extragenital sites and metastasize, suggesting greater malignance. The classification of TVT by cytology is a method that is simple, rapid and easy to accomplish, and permits the distinction between two TVT cell types, without necessitating more expensive exams that require specific equipment. Besides simple diagnosis, it becomes necessary to classify TVT into one of three morphological types (lymphocytic, plasmacytic or mixed), since there are two subclasses of TVT, whose morphology is reflected in the prognosis and resistance to treatment.

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