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RESIDUES OF PYRETHROIDS IN THE ADIPOSE TISSUE ADJACENT TO SPONTANEOUS MAMMARY CARCINOMA IN FEMALE DOGS

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ABSTRACT:

The exposure to environmental pollutants can be associated with the development of mammary cancer in female dogs. The objective was to identify pyrethroid alethrin, lambda-cialothrin, cypermethrin, deltamethrin and tetramethrin residues in the adipose tissue adjacent to the mammary gland with spontaneous carcinomas and correlate them with the morphology of the neoplasias. Thus, five grams of adipose tissue were collected from 30 female dogs and analyzed by the High Performance Liquid Chromatography exam. There was not a significant difference among the pyrethroids levels, the morphologic expression or the degree of malignity of the carcinomas analyzed by the Chi-square test.

Key Words: Female dogs; mammary tissue; carcinoma; pyrethroids.

RESUMO:

A exposição a contaminantes ambientais podem estar associados ao desenvolvimento de câncer de mama em cadelas. O objetivo foi identificar e quantificar resíduos dos piretróides aletrina, cipermetrina, deltametrina, tetramentrina e lambda- cialotrina no tecido adiposo adjacente a mama com carcinomas espontâneos e correlacioná-los com a morfologia destas neoplasias. Para isso, cinco gramas de tecido adiposo foram coletadas de 30 cadelas e analisadas por Cromatografia Líquida de Alta Eficiência. Não foi observada diferença

significativa entre os níveis de piretróide, a expressão morfológica e o grau de malignidade dos carcinomas analisadas pelo teste do Qui-quadrado.

Palavras-chaves: Cadelas; tecido mamário, carcinoma, piretróides

INTRODUCTION

The term agrotoxic, is used generically to describe natural or synthetic chemical substances, responsible for the combat of parasites on farms (CALDAS & SOUZA, 2000; JOBIM et al., 2010). Developed initially as an agricultural defense, now its use has been dispersed embracing domisanitary products, domestic animals parasite control, and public health campaigns. Though thoroughly distributed due to the practicability, easy access and economical importance, these substances are potentially toxic and their residues can contaminate the environment (PRESIBELLA, 2004; CERQUEIRA *et al.,* 2010).

Among the agrotoxic residues that can contaminate the environment, the pyrethroid group is prominent, in spite of being the most widely used pesticide in the world, little has been done to evaluate its potential as a hormonal deregulator (GAREY & WOLF, 1998; KASAT et al., 2002).They are listed as such by international agencies like Environmental Protection Agency (EPA), responsible for the regulation of chemical substances in the United States (EPA, 1997).

Man's exposure along with other animals to pyrethroid residues is mainly through ingestion of residues, and secondly by direct dermal contact or inhalation of suspended particles in the air (WOOLLEN et al., 1992; SODERLUND et al., 2002). The biotransformation of pyrethroids absorbed orally happens initially by the hydrolysis of the initial composition in the gastrointestinal treatment (SODERLUND et al., 2002; ANADON et al., 2009; YANG et al., 2010).

The metabolites preceding these processes are distributed heterogeneously way and deposited mainly in the liver, kidneys and adipose tissue interacting with the cellular representatives. In these organs, the metabolite levels decrease quickly, with exception to the adipose tissue due to lipophilic characteristics of the metabolites. The metabolism results in the formation of more polar compositions, excreted as such by the urine conjugated in form with endogenous substrates (taurine, glycine and PAPS) (SODERLUND et al., 2002; SANTOS et al., 2007).

Pyrethroids such as cypermethrin and the deltamethrin, are classified by the International Agency of Research on Cancer (IARC), as a level 3, in other words, an inconclusive carcinogen to man and the World Health Organization (WHO) defines deltamethrin as a moderately dangerous insecticide (IARC, 2008; EPA, 2008). The metabolites of the pyrethroids can present xenoestrogen characteristics, which is a substance that mimics the action of the feminine sexual hormone (FANG et al., 2001). For some authors the cypermethrin, tetramethrin and alethrin has been shown to induce the oxidative stress, generating active species of oxygen (AEO) in experiments with mice (KALE et al., 1999; GIRAY et al., 2001). The oxidative stress and the production of AEO may cause damage to DNA, acting as a chemical carcinogen responsible mainly for the uncontrolled growth of mesenchymal cells (ZEGURA et al., 2004).

However not all studies demonstrate that pyrethroids and their metabolites possess estrogenic activity (SAITO et al., 2000; KUNIMATSU et al., 2002; KIM et al., 2004). In

relation to the carcinogenicity, few reports are found in specialized literature on the subject. Though like other chemical substances of established performance, the occurrence of these effects depend on their molecular structure, the dose and the intensity of exposure (GARRIDO et al., 2005). In the current literature there is no work that relates the effect of pyrethroids in mammary tumors in female dogs.

The development of mammary spontaneous carcinoma in female dogs can involve feminine steroid hormones. These hormones are responsible for approximately 50% of all the neoplasic disorders in this species (MISDORP, 2002; GUIM et al., 2007; LANA et al., 2007). The importance of this type of neoplasia is due to the growth in the number of animals assailed by the disease in the last decade, as well as presenting likeness in several aspects to mammary cancer in women that rouses the scientific interest in the study using the female dog as an excellent experimental model for both species (ANTUOFERMO, 2007; LAFFIN et al., 2009; UVA et al., 2009; KOOISTRA et al., 2009).

The prognosis for patients with this type of neoplasia depends on the diagnosis necessary in the initial disease stage (ZUCCARI et al., 2001). The primary diagnosis of the tumor type is made by the cytopathologic exam (ZUCCARI et al., 2001; QUEIROGA & LOPES, 2002; PELETEIRO et al., 2006; RICK et al., 2009). The histopathological exam is the most appropriate method for the classification of mammary tumors in female dogs, especially in the characterization of malignant neoplasias (MISDORP, 1999).

According to the World Health Organization (OMS), tumors can be classified morphologically as benign, malignant, indifferent, hyperplasia and mammary dysplasia. Among carcinomas they are classified: in situ, complex, simple with tubulopapillary, solid, anaplastic subtypes and then there are the special that include the fusiform cells carcinoma, squamous cell, mucinous and rich in lipids (MISDORP et al., 1999). The degree of neoplasias is an attempt to evaluate its malignancy or aggressiveness, based on the

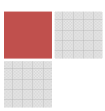
characterization of the tumor cells and the amount of mitoses (FONSECA & DALECK, 2000; LANA et al., 2007).

The etiology of the malignant mammary tumors in female dog is still not elucidated, but believed that it is related to its susceptibility to several factors of complex interactions, being external or internal to the organism and being both interlinked. The external causes are related to the environment and the habits of the animal (ZUCCARI et al., 2008; INCA, 2010).

The environmental effect is linked as a mammary carcinogen due to the vulnerability animals have to the artificial substances dispersed daily in the atmosphere. Among the residual pollution agrotoxics are the principal by-products in the industry as well as domestic garbage. According to the National Institute of Cancer (2010), 80% of the new mammary cancer cases in women are associated to environmental factors. The established proximity between the owner and pet determines the exposure level to the same environmental agents in both species (RIPLEY, 2001; ANTUOFERMO, 2007; JOBIM et al., 2010).

Considering the mentioned aspects, we become aware of the need for greater studies in pyrethroid performance as endocrine disruptores and possibly as cancerous agents. The objective of this work is to identify and to establish levels of residues of the pyrethroids alethrin, cypermethrin, deltamethrin, tetramethrin and lambda-cialothrin in the adipose tissue adjacent to the mammary gland of female dogs with and without carcinoma, so that one can discover any relationship of this pollutant in the mammary carcinogen.

MATERIAL & METHODS



In the present study, 30 female dogs were collected without predilection of race, with ages between five and 13 years, with clinical histopathologic diagnosis of spontaneous mammary carcinoma, assisted in the Veterinary Hospital of Faculdade de Medicina Veterinária e Zootecnia (FMVZ) of Universidade Estadual Paulista (UNESP), Campus of Botucatu/SP and in the Veterinary Hospital of Faculdade de Medicina Veterinária e Zootecnia de Garça/SP (FAMED), maintained by the Associação Cultural e Educacional de Garça/SP (ACEG).

In order to evaluate the corporal mass and determinate the physical state (Figure 1) the criteria described by HILL'S (1999) was used. The animals were classified as: thin, where the ribs are easily tangible, without fat covering. The base of the tail has prominent bones, abdominal curvature accentuated and silhouette of the waist quite accentuated. Ideal weight characterized by the easily tangible ribs, with little fat covering. The base of the tail with soft contour, tangible bones with fine fat layer and obese, when the ribs are difficultly tangible, with moderate to thick fat covering and the base of the tail arched.

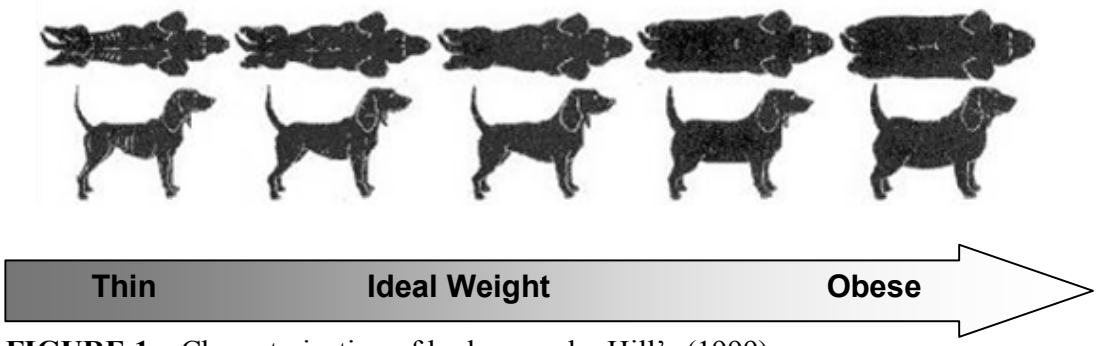


FIGURE 1 – Characterization of body mass by Hill's (1999).

After complete clinical exam of the animals and registration of data, the cytopathology exam was used to characterize preliminarily the increase in volume as a

neoplasia with mammary origin. In this procedure disposable hypodermic needles 13 x 4,5 mm were used to puncture mammary nodules smaller than 1,0 cm and 30 x 7 and 30 x 8 mm and for nodules with a diameter surpassing 1,0 cm disposable hypodermic syringes of 10 ml. The material obtained was distended on to hystologic slides. Part of the slides were fastened with methanol p.a. (Merck, Darmstadt, Germany) and ethanol 95% (Merck, Darmstadt, Germany) and stained by May-Grünwald Giemsa's techniques and Papanicolaou, respectively. The samples were examined in the light microscope (ZEISS - I Model AXIO Imager A1).

The animals with positive cytopathologic diagnosis of mammary neoplasia were submitted to a partial or radical mastectomy. The surgical pieces were directed to the Service of Veterinary Pathology of FMVZ/UNESP, campus of Botucatu for analysis anatomopathologic and material processing for histopathology exam. The fragments of carcinomas were fixed in tamponade formalin 10%, processed routinely and stained with Hematoxylin and Eosin. The material was read in light microscope (ZEISS - I Model AXIO Imager A1) and classified following the criteria adopted by the World Organization of Health (MISDORP et al., 1999).

Five grams of the adipose tissue adjacent to the carcinomas was removed, frozen at a temperature of – 4º C until the analysis of the quantitative High Performace Liquid Chromatography (HPLC) technique, according to the method of BISSACOT and VASSILIEFF (1997) modified for processing these sample types. For analysis, the model CG 480C was used from CEATOX, Institute of Biosciences - UNESP/Campus of Botucatu-SP. In order to read the levels of pyrethroids an ultraviolet detector, model CG 435B with a wavelength of 266nm, was used integrating the pattern of the peaks with attenuation of 2.0 and inclination of 300 with a speed of 10mm/min.

RESULTS & DISCUSSION

This work identified and quantified the alethrin, cypermethrin, deltamethrin, tetramethrin and lambda-cialothrin levels in the adjacent adipose tissue of the mammary gland in female dogs with the HPLC technique, following the same method used by ANDRADE (2008). The analysis demonstrated sensibility in the quantitative and qualitative determination of the residues, proving its efficiency and adaptability in the analysis of different substances as described by other authors (BISSACOT & VASSILIEFF, 1997; ANDRADE, 2008).

The corporal weight of the animals was between 3,3 and 40 kg, eight animals were obese (26,7%); 21 regular (70%) and only one was classified as thin (3,3%). The analysis of the adjacent adipose tissue of the mammary gland, in the present study, demonstrated that the animals presented varied levels of residues of pyrethroids regardless of the classification of corporal mass. The largest pyrethroid indexes were detected in animals considered regular, followed by thin and lastly obese. Although the number of studied animals is small, the quantification of these pyrethroids can demonstrate that the distribution of residues occurs in a non-homogeneous way in the different tissues, as related by CRAWFORD et al. (1981); VIRAN et al. (2003) and ANADÓN, et al. (2006).

The lipophilic influence of some pyrethroid metabolites can be linked to the deposition of a larger concentration in the adipose tissue (CRAWFORD et al., 1981; VIRAN et al., 2003). In thin animals, even if in smaller amounts, the residues come in larger concentrations, due to the low volume of available adipose tissue in the body of the animal. This doesn't happen in obese animals, although that supposition should be confirmed with greater study.

The cytopathology and histopathology exams were used in the experiment, respectively, in the screening of animals and identification of the tumor, as described by Zuccari et al. (2001); Queiroga & Lopes (2002); Peleteiro et al. (2006); and in the

morphologic classification and expression of the cellular criteria of malignancy as suggested by Misdorp, (1999); Karayannopolou et al. (2005); Cavalcanti & Cassali (2006).

The malignant neoplasias found with larger incidence were complex (56,6%) (Figure 4 and 5), followed by simple (43,4%) (2 and 3) with its subclasses. Contrary to the frequency found by Burini, (2002); Oliveira, (2003); Hataka, (2004); relating simple carcinoma, followed by complex carcinoma.

The use of these exams together demonstrated to be an important tool in the diagnosis routine and research due to the low invasion degree in the collection of samples of the cytopathology and the amount of information obtained through histopathology, as described by other authors (CAVALCANTI & CASSALI, 2006; RICK et al., 2009).

Afterward, the carcinomas were classified by their degree of malignancy I, II and III (Table 1).

TABLE 1 – Carcinomas histopathologic classification and graduation according to the degree of malignity.

Histopathologic Classification	**n**	**Degree I**	**Degree II**	**Degree III**
Simple Carcinoma	13	4 (13,3%)	4 (13,3%)	5 (16,7%)
Complex Carcinoma	17	1 (3,3%)	7 (23,3%)	9 (30%)
X^2=3,867				**P=0,1447**

There is no difference observed (p>0,05) by Chi – square test.

In the present study a significant difference in relationship of the degree of malignancy in different carcinomas and the levels of pyrethroids found in the adipose tissue adjacent to the tumor was not observed.

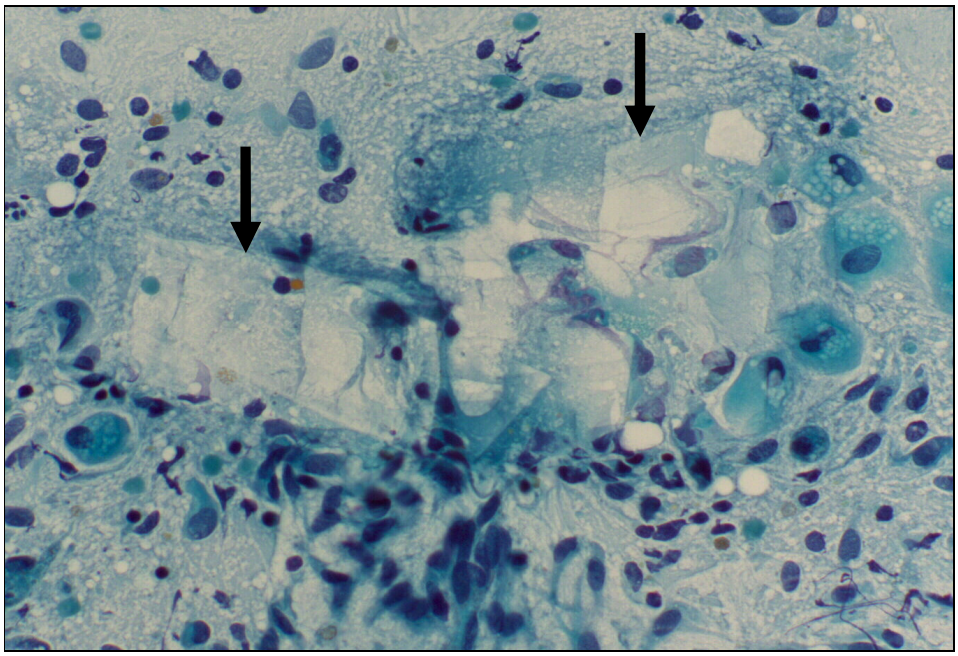


FIGURE 2 – Cytopathological exam: female dog simple mammary carcinoma, the presence of cholesterol crystals is noted (arrow) among epithelial neoplasic cells. Papanicilau,40X.

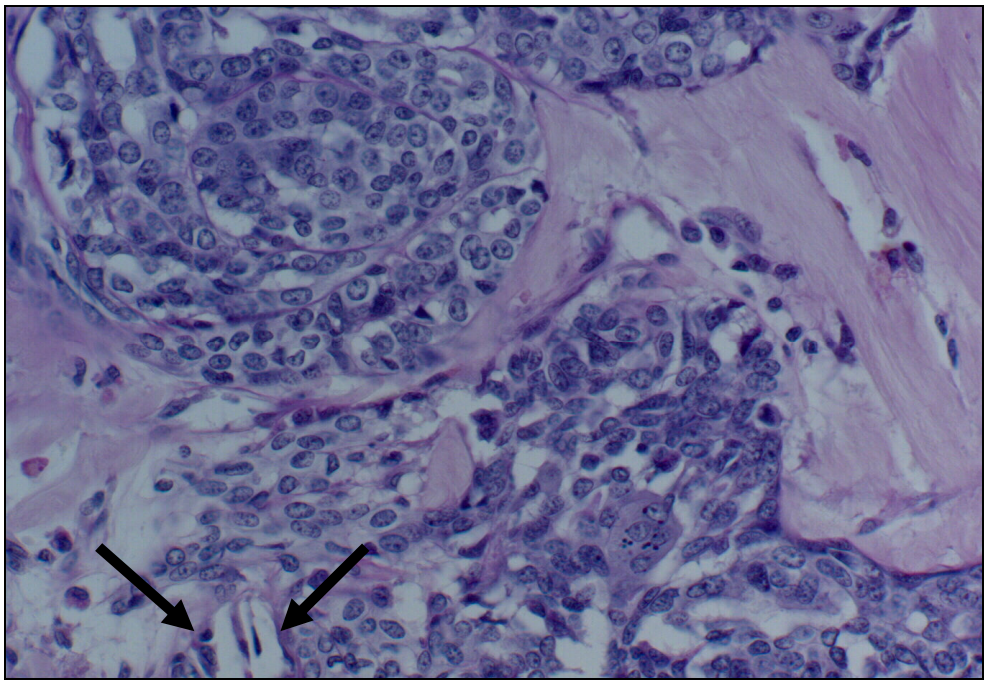


FIGURE 3- Histopathological exam: female dog simple mammary carcinoma, the presence of cholesterol crystals is noted (arrow) among epithelial neoplasic cells, forming alveolar arrangements. H.E., 40X.

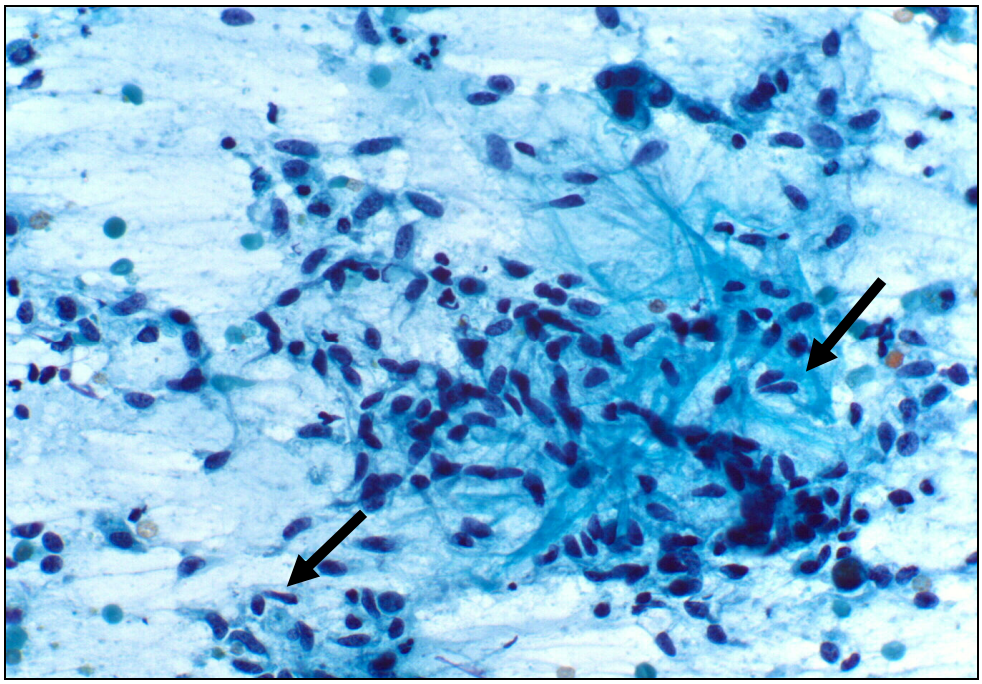


FIGURE 4 - Cytopathological exam: female dog complex mammary carcinoma, epithelial and mesenchymal cellular components, presence of fusiform cells that look like myoepiteliais (arrow). Papanicolau, 20X.

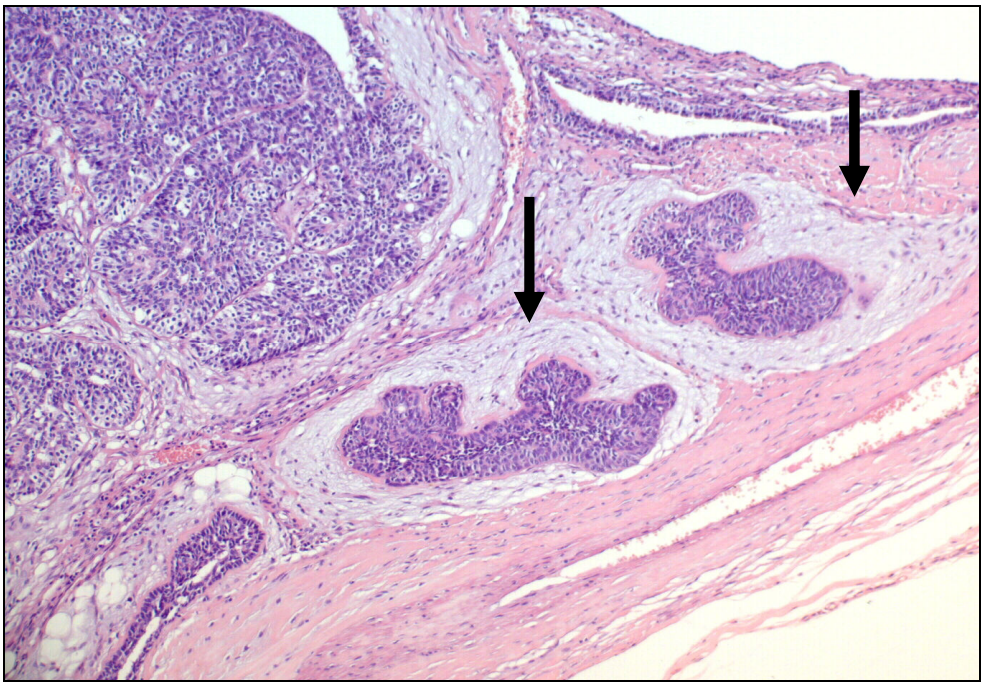


FIGURA 5 - Histopathological exam: female dog complex mammary carcinoma; acinar proliferation of epithelial neoplasic cells, involved by amorphous mixed tissue (arrow). H.E, 20X.

The carcinomas were located in one or multiple mammary glands. When the masses were located in just one area (28/30), the most affected mammary glands were the abdominals (50%), followed by the mammary inguinal (23,3%) and thoracic (20%). When the masses were multiple (02/30), the lesions prevalence was observed on the left abdominal area (3,3%) and by the whole right side extension of the mammary glands (3,3%).

In relation to the location of the studied neoplasias, the larger predilection was observed by the inguinal and abdominal mammary glands. The data found is similar to that described by other authors (QUEIROGA & LOPES, 2002; OLIVEIRA et al., 2003; HELLMÉN, 2005). The reason for which these glands are attacked more is possibly due to the larger amount of mammary parenchyma, suffering a larger proliferative response due to

the effects of estrogen. The sensibility and the exposure to cancerous chemicals elevates the risks of developing mammary cancer (NARDI et al., 2008), though in the present study no differences were observed with significant relationship to the location of the tumors in the mammary tissue and the amount of residues of detected pyrethroids in the adjacent adipose tissue (Table 2).

TABLE 2 – Comparison among frequency, location of the mammary carcinoma and the corporal mass.

Localization	**Corporal Score**		
	Thin	**Obese**	**Ideal Weight**
Right abdominal cranial	0	2	5
Left abdominal cranial	0	0	3
Right abdominal caudal	0	1	1
Left abdominal caudal	0	2	2
Right inguinal	1	0	3
Left inguinal	0	2	1
Multiple in entire right mammary chain	0	0	1
Left thoracic Abdominal	0	0	1
Right thoracic Caudal	0	0	1
Left thoracic Caudal	0	0	1
Right thoracic Cranial	0	0	1
Thoracic Caudal	0	1	1
X^2=15,221			**P=0,8526**

There is no difference observed (p>0,05) by Chi – square test. The attacked mamma doesn't depend of the corporal score.

The comparison between the concentration of pyrethroid residues and the morphologic expression of carcinomas diagnosed by the histopathology exam didn't demonstrate significant differences (Table 3), however the largest prevalence was verified in the compound carcinomas in relation to the simple.

TABLE 3 – Comparison among the pyrethoids levels diagnosed in carcinomas

Neoplasias	Pyrethroids residue presence		
	Presence	No presence	N
Simple Carcinoma	5	8	13
Complex Carcinoma	11	6	17
X^2=2,038		**P=0,533**	

There is no difference observed (p>0,05) by Chi – square test. The attacked mamma doesn't depend of pyrethroids residues presence.

That proliferation of the mesenchymal component in the complex carcinoma might have been a consequence of pyrethroid residues' influence that was deposited in the tissue. The metabolism of the pyrethroid residues, as well as other chemical substances, occurs by the action of a variety of enzymes, that can alter the expression of some genes when they act in an exacerbated way, turning the metabolites into eletrophilic chemicals, allowing them to link to composed DNA covalents, acting as an indirect chemical carcinogen (IARC, 2008).

Another way that the chemical residues of the pyrethroids can be corroborating in this process, could be related to oxidative stress, which happens during the disintoxication process of tissues. Consequently this process occurs in the production of AOE, responsible for the I stimulate of cellular multiplication, mainly mesenchymal cells in different organs such as the liver, kidney, mediastine and also in the mammary gland (ZEGURA et al., 2004).

The concentration of the pyrethroids in the tissue adjacent to the spontaneous mammary carcinomas in female dogs although it has not demonstrated a significant influence in the development of mammary neoplasias or its performance as a direct or indirect carcinogen, its action in the organism during lingering periods of exposure is not conclusive (SHEETS et al., 2004; SHAFER et al., 2005; ANADON et al., 2009). Recent studies indicate that exist enough evidence for us to study with greater attention the

performance of these residues compositions and performance of such in different animals (GAREY & WOLFF, 1998; CHEN et al., 2002; ZHAO et al., 2008; YANG et al., 2010).

CONCLUSIONS

Larger indexes of complex carcinoma were observed in animals that presented pyrethroid residues in the adjacent adipose tissue of the mammary gland. The study demonstrated that the dog can serve as a biological model in the identification and quantification of environmental residues that can be involved in the development of cancer including the mammary gland.

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