Imaging and laboratory diagnosis of mammary pathology in the bitch

SUMMARY OF PhD THESIS

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1 LITERATURE REVIEW

The literature review concentrated on highlighting the characteristics of canine mammary anatomy, histology and physiology that explain the physiopathology of mammary lesions, as well as offer the physical explanation of images obtained using medical imaging techniques. It focused on the formation and development of the mammary gland, the mammary morphology and histology, its vascularization and innervation and the physiology of lactation. Subsequently, there have been identified the most important lesions that affect the mammary gland during and outside lactation.

After the establishment of the main mammary lesions, as described by the literature, there have been investigated the imaging methods used in their diagnosis, the research focusing on sonography, radiography, thermography, computed tomography scans and magnetic resonance imaging. This section wanted to identify the utility and limitation of imaging methods of diagnosis used in canine mammary pathology.

One last section of the literature review researched the mathematic means of analyzing medical images of mammary tumors, especially ultrasound images.

2 PERSONAL CONTRIBUTION

2.1 AIM OF RESEARCH

Today, imaging methods are more and more involved in the evaluation of health status of companion animals, but they are not as spread and used in veterinary medicine as they are in human medicine, at least in our country.

Both in our country, as at global level, the pathology of the mammary gland in the bitch are an important part of all pathologies diagnosed in this species. Because of the similarities between different mammary diseases, often there is the need to use complementary means of diagnosis in order to make the best therapeutical decisions. Medical imaging is suitable for the examination of this anatomical segment, but there is a need of a standardized diagnostic plan and doctors specialized in this field in order to get the best results (Lucas, 2014).

The aim of this research is to highlight the imaging characteristics of different lesions of the mammary gland of the bitch in order to increase the diagnostic importance of imaging investigations of this organ, nationwide. This wants to be an integrated study that combines clinical examination with imaging examination and, where it is possible, with laboratory exams. As well, the study wants to offer references both to researchers in the medical diagnosis field, and to clinicians that face this kind of lesions very often. That is why the experiments were adapted in order to best mimic
the conditions encountered in veterinary clinics, in order to increase the applicability of the results.

2.2. MATERIALS AND METHODS

The research was conducted during April 2013-June 2015 in the Clinic of Pathology of Reproduction from the Faculty of Veterinary Medicine Cluj-Napoca and four other partner clinics (Clinic of Faculty of Veterinary Medicine, Bucharest, and three private practices from Brașov) and February-May 2015 in the University Veterinary Hospital of Murcia University, Spain. The biologic material consisted of bitches with physiologic lactation or clinical signs of a mammary lesion when presented to consult.

For each animal the anamnesis was noted and a clinical exam was done, according to the clinical examination chart designed for this research, followed by an imaging exam using at least one method of the following: echography, radiography, thermography or using computer tomography. Also, the sonography images of neoplastic formations collected using the ultrasound machine of the Clinic of Pathology of Reproduction from the Faculty of Veterinary Medicine Cluj-Napoca (Mydray DC3Vet) were mathematically analyzed in order to distinguish different tumors. After the examinations, biological samples were collected, where possible, consisting of milk samples for animals with physiologic lactations, nervous lactations and mastitis, respectively tissue samples for the ones with mammary tumors.

The data collected was statistically interpreted and graphically represented using Microsoft Office Excel® with Analysis Tool pack add-on and Origin 8.5.1®. The mathematic analysis of echography images was done using ImageJ® software and Fraclac® plug-in.

2.3. RESULTS AND DISCUSSIONS

2.3.1. The organization of experimental groups

During the research, 70 bitches corresponded to the inclusion criteria. Other six animals with mammary lesions were also consulted, but were excluded because the owners refused the collection of biological samples.

Out of these animals, ten were healthy, seven had nervous lactation, six had mammary cysts, 16 were diagnosed with mastitis and 32 had mammary tumors. Seven animals (10%) presented two mammary lesions at the same time, thus were included in both groups. The proportion of each diagnosed pathology of the bitch included in the study can be seen in Fig. 1. Four animals were clinically healthy, but the laboratory analysis diagnosed them with subclinical mastitis.

The age of the animals in this research varied between 10 months and 14 years. Fig. 2 represents the distribution of each mammary lesion according to age. It can be observed that all animals diagnosed with physiological lactation were young (average of 4.3 years), as were the ones diagnosed with mastitis. Mammary tumors had a higher incidence at older ages. Nervous lactations and mammary cysts were diagnosed at all
ages. For all researched lesions, the mammary glands most affected were the posterior abdominal and inguinal ones.

The main mammary lesions diagnosed were mammary tumors, this result being in accordance with the specialty literature (Lucas, 2014; Im et al., 2014; Grünzig et al., 2015), presenting an incidence as high as 50% of intact bitches. The literature does not offer much data regarding the incidence of mammary cysts and mastitis, but presents as many as 87% of bitches suffering of nervous lactation at least once in their lifetime (Janssens, 1986). The low incidence of these lesions in our study can be explained by the hesitation of owners to get to the veterinarian unless there are complications.

The step increase of mammary tumors incidence after the age of nine years is in accordance with the specialty literature (Hellmen et al., 1993; Chang et al., 2009; Petrov et al., 2014). The present research reports mastitis evolving at a low age, often at the first lactation. There have not been identified other studies that present the average age of mastitis onset. The incidence of nervous lactation and mammary cysts did not appear to be affected by age, probably because these lesions are recurrent, thus after the first episode, they can be diagnosed at the next sexual cycles.

2.3.2. Echography diagnosis of the non-neoplastic pathology of the bitch mammary gland

The echography exam showed the characteristic architecture of the healthy mammary gland, the image being composed from skin, mammary parenchyma and muscles. Mammary glands with nervous lactation presented similar features, being impossible to distinguish them by sonography. The mammary cysts also showed characteristic aspects, with multiple cystic formations in the mammary parenchyma and vascularity being present only in the intercystic space.

The inflamed mamma did not present the architecture described above, being difficult or impossible to differentiate the different tissues. The bitched diagnosed with subclinical mastitis presented the same aspects. 66% of the animals presented physiologic aspects at the periphery of the mammary gland, but not in the center. Only 16.21% of the inflamed mamma kept the echogenicity of the healthy tissue, 37.8% being hypoechoogenic and 45.16% being hyperechogenic. As well, 74.19% were heterogeneous. In eight mammary glands (25.81%) there have been acoustic artefacts: reverberation (75%), posterior attenuation (12.5%) and posterior enhancement.
The reverberations were associated with Gram- infections. The vascularization was absent in 12.91% of cases, all mammary glands presenting gangrenous mastitis.

The aspects observed for the healthy mammary gland are in accordance with the specialty literature (Trasch et al., 2007). The mammary cysts proved easy to diagnose, but when lesions are extended it can be confused with neoplasia, results similar to the ones found in women (Berg et al., 2003). The loss of normal architecture by the inflamed mammary glands was also presented by the literature (Trasch et al., 2007), but some aspects observed in this research were different from other studies. There has not been identified any study that describes the diagnosis of subclinical mastitis using ultrasonography in the bitch. Impairment of mainly the central area of the mammary gland is in accordance with other studies that claim that the main pathway of contamination is ascending (Schäfer-Somi et al., 2003). The literature describes a decrease in the echogenicity of the mammary parenchyma of bitches with mastitis (Trasch et al., 2007), but not its increase. Hyperechogenic mammary tissue was described in other species, especially ruminants, when chronic mastitis is present (Santos et al., 2015). The physiopathology mechanisms of chronic mastitis would explain the physics for forming a hyperechogenic image. The presence of reverberations in echography images of inflamed mammary glands has been reported before (Trasch et al., 2007), but it has not been linked to the bacteria responsible for the inflammation. The absence of vascularization in the inflamed mammary gland proved to be an aggravating element, being characteristic to gangrenous mastitis, as literature shows (Trasch et al., 2007).

### 2.3.3. Imaging diagnosis of mammary tumors of the bitch

During the research there have been identified and diagnosed 32 females with mammary tumors, 26 of which met the requirements of the study. From these, five were diagnosed with benign tumors and 21 with malignant ones. The total number of tumors was 71, five benign and 66 malignant.

The results obtained after the echography exam, for benign and malignant tumors, can be seen in table 1.

<table>
<thead>
<tr>
<th>Observed property</th>
<th>Benign tumors</th>
<th>Malignant tumors</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumors</td>
<td>5</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oval</td>
<td>100%</td>
<td>42.42%</td>
<td>0.045*</td>
</tr>
<tr>
<td>Spherical</td>
<td>0%</td>
<td>37.88%</td>
<td></td>
</tr>
<tr>
<td>Polyhedral</td>
<td>0%</td>
<td>19.7%</td>
<td></td>
</tr>
<tr>
<td>Shape of the edge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regulated</td>
<td>80%</td>
<td>53.3%</td>
<td>0.243</td>
</tr>
<tr>
<td>Unregulated</td>
<td>20%</td>
<td>46.7%</td>
<td></td>
</tr>
<tr>
<td>Clarity of the edge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear</td>
<td>100%</td>
<td>39.4%</td>
<td>0.008**</td>
</tr>
<tr>
<td>Unclear</td>
<td>0%</td>
<td>60.6%</td>
<td></td>
</tr>
<tr>
<td>Capsule</td>
<td>0%</td>
<td>12.12%</td>
<td>0.409</td>
</tr>
<tr>
<td>Invasivity</td>
<td>0%</td>
<td>51.51%</td>
<td>0.026*</td>
</tr>
<tr>
<td>Echogenicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechogetic</td>
<td>100%</td>
<td>84.84%</td>
<td>0.643</td>
</tr>
<tr>
<td>Izoechogenic</td>
<td>0%</td>
<td>9.1%</td>
<td></td>
</tr>
</tbody>
</table>
Out of the studied parameters, only the shape of the tumor, the clarity of the edge and the invasive character of the tumor were statistically significant for differentiating benign from malignant tumors. Nevertheless, none of these parameters considered alone can differentiate all benign tumors from malignant ones.

The percentage of malignant tumors that had regular shapes and margins was higher in this study than in other studies (Gonzales et al., 1997; Nyman et al., 2006b; Baștan et al., 2009), probably because many of the analyzed tumors had small sizes, being in the first stages of development. This aspect excludes the shape of the tumor as a mean of differentiation, especially for small tumors. The proportion of malignant tumors with heterogeneous aspect was similar to the one reported by other studies, but the benign tumors were also heterogenic. The vascularization of tumors presented different characteristics from the ones presented by Nyman et al. in 2006, who considered central vessels characteristic for benign tumors, and peripheral ones characteristic for malignant tumors (Nyman et al., 2006a). The differences between the results of this study and the ones found in the specialty literature can be explained by the high variability of mammary tumors and by the failure to determine the exact age of a tumor.

One female with a malignant tumor was also examined using SonoVue contrast agent for sonography. The images obtained highlighted better the heterogenic aspect of the tumor and the aspect of lymph nodes. Radiologic and CT exams without contrast agent did not bring new information regarding the type of tumor.

### 2.3.4. Thermography diagnosis of canine mammary tumors

After taking thermography images to six clinically healthy animals, there could be seen large variations of local temperature between mammary glands of the same mammary chain, for the same animal, but only variations under 1°C for mammary pairs. This was true for axillary and inguinal lymph nodes as well. The results of the bitches that were diagnosed with mammary tumors can be seen in Fig. 3.
In all cases, the mammary glands that had neoplastic lesions had the surface temperature over 1°C higher than its pair of the other mammary chain. The differences were evaluated by comparing their modules, as well as their average and the sum of squares, parameters that were statistically significant. These results were in conformity with the specialty literature (Mocanu and Miclău, 2005).
2.3.5. Computerized analysis of echography images of mammary tumors

There have been analyzed 12 images representing benign tumors and 44 representing malignant tumors, all collected with the same ultrasound machine and the same image adjustments. The mean values obtained through mathematic analysis of images for the two tumor types are presented in table 2.

Table 2

<table>
<thead>
<tr>
<th>Observed property</th>
<th>Benign tumors</th>
<th>Malignant tumors</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>1.00</td>
<td>2.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>0.56</td>
<td>1.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>0.54</td>
<td>3.24</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean of grey shades</td>
<td>30.09</td>
<td>37.71</td>
<td>0.144</td>
</tr>
<tr>
<td>Standard deviation of grey shades</td>
<td>15.38</td>
<td>19.22</td>
<td>0.032</td>
</tr>
<tr>
<td>Fractal dimensions</td>
<td>1.13</td>
<td>1.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunarity</td>
<td>0.11</td>
<td>0.16</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Although only two of the parameters were not statistically significant for the differentiation of benign tumors from malignant ones, none of these can completely separate the neoplastic formations, because the variation intervals of the two tumoral types overlap. For this reason the relationship between these parameters was researched, as well as the possibility to use them together in order to establish a diagnostic. Thus, it was opted for the use of a multiple logistic binominal regression that included tumor area ($x_1$), standard deviation of grey shades ($x_2$), fractal dimension ($x_3$) and lacunarity ($x_4$). The equation that best describes their relationship for establishing a diagnosis is:

$$y=-606.1039+x_1*53.1883676+x_2*6.921819617+x_3*309.8021732+x_4*663.4483074$$

After using this formula, the two groups of images completely separated, the benign tumors having negative values, while the malignant ones had positive values. The results were statistically significant ($p<0.001$). There have not been identified any similar studies done on canine mammary tumors, but the results were compared with studies made in human medicine on breast cancer, studies that also identified fractal dimension, lacunarity and histogram variation as elements for differentiating tumors (Shi et al., 2010).

2.3.6. Conclusions and recommendations

The results of the presented research allow the creation of a global vision of the subject, emphasizing the following conclusions:

- the most important canine mammary lesions, from the incidence point of view, are tumors, followed by mastitis;
- the ultrasound examination is easy to use and offers important information regarding the aspect of lactating mammary parenchyma and mammary tumors;
- the healthy mammary gland has a specific architecture composed of skin, glandular parenchyma and muscle tissue, architecture that is lost when there are inflammations, including subclinical ones;
ultrasound exam in B mode proved efficient in separating chronic mastitis from acute ones;

the absence of vascularization in the inflamed mammary parenchyma is an indicator of its necrosis, being an ominous indicator;

the shape, margin clarity and invasive character of tumors are parameters statistically significant in differentiating benign tumors from malignant ones;

when it is correlated with clinical examination and other laboratory exams, thermography is a useful imaging method for the evaluation of the evolution of neoplastic lesions and lymph node reaction;

temperature differences between the two mammary chains, read using thermography, expressed as individual values, average or sum of squares, are the best indicator of the evolution of a neoplastic lesion;

the equation \( y = -606,1039 + \text{area} \times 53,1883676 + \text{standard deviation of grey shades} \times 6,921819617 + \text{fractal dimension} \times 309,8021732 + \text{lacunarity} \times 663,4483074 \) best defines the correlation between these parameters;

using the formula of regression, the two types of tumors were completed separated.

**Recommendations**

We recommend the use of ultrasound as screening exam of all lactating females for the quick diagnosis of mammary gland pathologies, before the impairment of offspring or the female.

We recommend the use of thermography as a complementary method of diagnosis and screening of canine mammary tumors. As indicators of lesions we recommend the use of temperature differences between mammary pairs.

We recommend making a study to calculate sensitivity, specificity, positive prediction value and negative prediction value for the regression equation obtained in this study.

**BIBLIOGRAPHY**

1. LUCAS XIOMARA, 2014, Patología y farmacología mamaria, in Medicina y Cirugía del Sistema Endocrina y de la Reproducción, Ponencias y Comunicaciones, Madrid 13-15 de Marzo, p. 97-102
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15. BAŞTAN, A., ÖZENÇ, E., YAĞCI, İ. P., ACAR, D. B., 2009, Ultrasonographic evaluation of mammary tumors in bitches. *Journal of the Faculty of Veterinary Medicine, University of Kafkas, Kars (Turkey)*.
