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B-Mode and Doppler Sonography of the Mammary Glands in Dairy Goats for Mastitis Diagnosis

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Contents
This study aimed to evaluate the sonographic characteristics of the udder and teats and to determine the Doppler indexes of mammary artery in healthy and undergoing subclinical and clinical mastitis goats. Thirty animals among Saanen and Alpine Brown goats were arranged in three groups, healthy goats (HG), goats with subclinical mastitis (SMG) and goats with clinical mastitis (CMG). Using the B-mode, the sonographic characteristics (echotexture and echogenicity) and biometry (diameter and area of the udder cistern, diameter and area of the teat cistern and thickness of the teat wall) were evaluated. Using Doppler ultrasonography, the vascular indexes of the mammary artery were obtained. It was observed hyperechogenicity with solid component in the gland cistern when comparing animals with clinical mastitis and healthy mammary tissue. Regarding the echotexture of the breast tissue, there was heterogeneity in the mammary parenchyma on the three groups, for the milk, it was observed homogeneity for animals on HG and SMG and heterogeneity for animals on CMG. Grey-scale quantitative assessment revealed increase in echogenicity (mean value) for all the structures when comparing the three groups. Biometry did not reveal statistical difference between groups, for none of the evaluated structures. Doppler examination of the mammary artery showed the decrease of end diastolic velocity and raise of pulsatility index between groups. The association of B-mode and Doppler ultrasonography is useful for the evaluation of the udder of dairy goats with mastitis. It is a sensitive and specific method for the study of this disease. Doppler mode was unable to establish reliable criteria for diagnosis of subclinical mastitis. Moreover, the quantification of echogenicity is a useful technique for the evaluation of the milk in animals with mastitis; therefore, it is suggested that it can be used as complementary technique for the diagnosis of mastitis in goats.

Introduction

Conventional ultrasonographic imaging is a non-invasive diagnostic method that allows for the repeated and safe evaluation of various regions of the internal organs (Simões 2008). The application of this technique to examine the mammary gland has been evaluated in several animal species of veterinary interest (Kirk and Glenn 1996; Flöck and Winter 2006; Melo et al. 2012). The Doppler technique provides a means to assess the vascular indices of blood flow that can aid in the differential diagnosis of the primary mammary gland disorders (Feliciano et al. 2012). In goats, however, the Doppler sonography has only been employed to assess blood flow velocity in the milk vein (Nielsen et al. 1990).

Considering the potential applicability of both the grey-scale and Doppler imaging modalities for the diagnosis of mammary gland disorders, the sole objective of this study was to determine the B-mode and Doppler sonographic characteristics of the mammary gland in healthy, dairy goats as well as in dairy goats undergoing mastitis in both the subclinical and clinical forms and assess if they are related to the disease, specially the subclinical form for being of difficult diagnosis.

Materials and Methods

The present study was carried out in the College of Agriculture and Veterinary Sciences of the São Paulo State University facilities, located in the city of Jaboticabal (latitude 21°15′17″S and longitude 48°19′20″W, 605 m above the sea level), São Paulo State, Brazil. Climate of this region is classified as mesothermal subtropical, with mean annual temperature of 22°C.

Thirty goats, Saanen (26) and Alpine Brown (4), aged between 2 and 4 years, multiparous, fed with commercial balanced ration, coast cross hay, mineral salt and water *ad libitum* were examined 7 days post-partum and were arranged in three groups of 10 animals each: health goats (HG), goats undergoing subclinical mastitis (SMG) and goats with clinical mastitis (CMG). The animals were manually milked twice a day and the samples were obtained before the first milking; therefore, the udders were full, also the goats had no contact with their offspring.

Careful clinical examinations were performed according to Pugh (2005). Strip cup test and California Mastitis Test (CMT) were used to evaluate the milk, also, its characteristics were evaluated according to criteria collated by Quadros (2007). The ultrasonograms were obtained with the MyLabTM30 VET (Esaote S.p.A., Genova, Ligúria, Italy) with a microconvex multifrequency (5–7.5 MHz) probe, frequency used was 6.6 MHz. To perform B-mode examination, the probe

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was positioned directly on the udder and teats. Only ultrasound gel was used to improve images quality. Scanning depth varied between 3 and 4 cm for both B-mode and Doppler techniques.

Using the B-mode ultrasonography, the echotexture of the mammary gland parenchyma as well as milk echogenicity, diameter and area of the gland cisterns, teat cistern diameter and surface area of the mammary structures were determined. The main gain was set up to 52% of a maximum value and a single focal point was positioned to the regions of interest; all settings including the near and far gain and contrast were kept constant throughout the examination period.

Computerized image analysis of the mammary gland ultrasonograms utilized commercially available image analytical software (Image ProPlus®; Media Cybernetics Inc., San Diego, CA, USA). On the digital images, polygon metres were used to delineate the areas of interest (sinuses, alveoli and parenchyma). The mean numerical pixel values (NPVs), pixel heterogeneity (standard deviation of NPVs) as well as maximal and minimal pixel values from each region of interest were computed. Grey-scale values obtained for multiple alveoli and parts of the parenchyma were then averaged to give the overall mean of the respective mammary glands.

To locate the mammary artery, the probe was positioned medially on the caudal region of the udder, near udder's insertion to the abdomen. Using colour mapping, the mammary artery was identified and located. For the spectral Doppler, the axis of the sound beam and the axis of the mammary artery were parallel to each other, but the insonation angle not exceeding 60°. To make the mammary artery identification easier and the waveforms evaluation more precise, the power Doppler function was employed. It also increases the sensitivity of the blood flow measurements and transforms the examination from angle independent to insonation or incident angle. Moreover, it was performed the analysis of maximum power in pulsed Doppler and the smallest Doppler wall filter for the characterization of the whole signal amplitude. A gate between 2 and 3 mm (equivalent to 2/3 of the vessel's diameter) was positioned in a central area of the vessel with apertures to measure the spectral trace of flow, spectral curve and vascular index, which was obtained automatically following software identification of the ultrasonic scanner for each waveform. A minimum of three subsequent waves were obtained to perform its evaluation. With the waveforms without artefacts and after the adjustment of the angle of insonation, the image was frozen, proceeding to morphological analysis of the waves. After that, the measurements of vascular indices on mammary artery were performed. Pulsed Doppler was used to determine the systolic peak velocity (SPV), end diastolic velocity (EDV), vascular resistance index (RI = [SPV−EDV]/SPV) and pulsatility index (PI = [SPV−EDV]/mean velocity) of the artery. The indices listed above were determined automatically (Feliciano et al. 2012).

Results

The clinical evaluation revealed no abnormalities on goats of HG and SMG. The milk also had normal characteristics. Goats of CMG group had elevated udder temperature, when compared to the body's temperature, both evaluated by palpation. The mean rectal temperature of this group was 41.5°C, and they also expressed pain when the udders were manipulated. The milk was yellowish and with dense aspect. Majority of the animals of this group had also oedema and nodules in the udder and were apathetic.

Strip cup test revealed flocs in the milk of the animals in CMG and no changes for the other two groups. For CMT, it was considered the three crosses reaction as indicative of clinical mastitis. Both tests are shown in Fig. 1.

In the grey-scale ultrasonograms, it was possible to visualize the hyperechoic mammary parenchyma with slightly coarse echotexture and to detect the presence of non-echogenic content (milk) in the cistern region showing as echogenic spots for healthy animals. The echotexture of the mammary parenchyma was heterogeneous in all three groups, the exception being the milk, which was homogeneous in groups HG and SMG and heterogeneous in CMG group. Goats of the CMG group had hyperechoic mammary parenchyma with presence of solid component (flocs) in the gland cistern. Ultrasonographic differences between healthy and sick animals are shown in Fig. 2.

Quantitative analysis of the pixel intensity revealed that mean pixel values increased between groups, being lower for healthy animals and higher for animals of the CMG group. The only exception was the standard deviation and heterogeneity of the milk in the teat cistern. Quantitative echotextural attributes of the mammary gland and teats have been summarized in Table 1. Number zero represents the black colour and number 255, the white colour. Standard deviation is the pixels heterogeneity.

Morphometric analyses of the caprine mammary glands in this study did not reveal significant differences between groups (Table 2).

Doppler evaluation of the mammary artery revealed significant differences between groups. The EDV value was higher for HG than SMG and CMG, respectively. The contrary was observed for PI values, which had the lowest value on HG group and the highest on CMG (Table 3).

Discussion

Clinical evaluation of the animals was performed as recommended by Pugh (2005). Goats undergoing subclinical mastitis presented no abnormalities in clinical examination, as expected. The whole symptomatology observed in goats from CMG is in accordance with the cited by Kirk and Glenn (1996). The interpretation of the CMT is based on visual observation of the milk after

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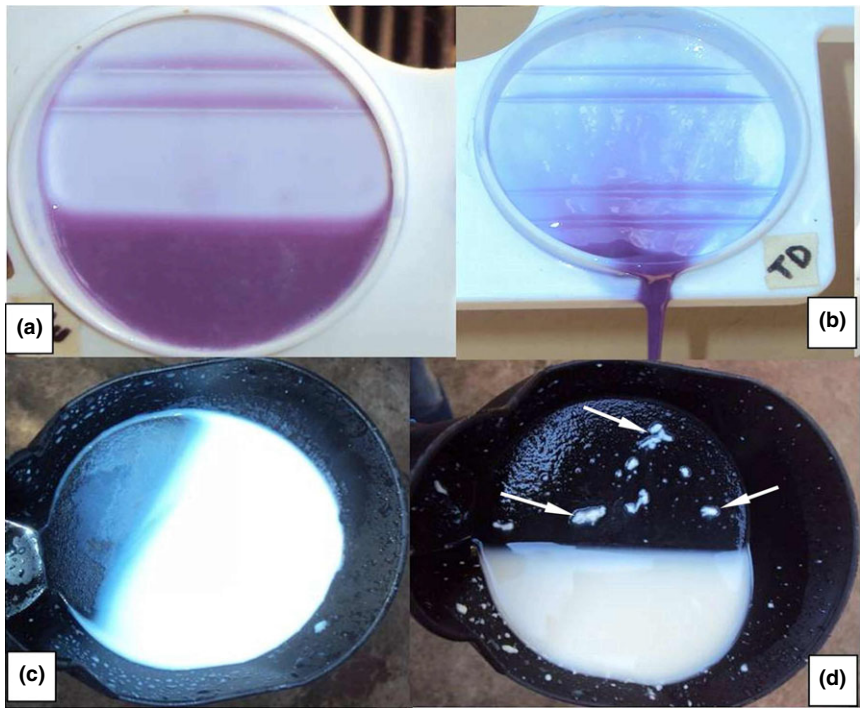

Fig. 1. Goats' milk samples for California Mastitis Test (CMT) and strip cup test. (A) negative CMT; (B) three crosses CMT reaction (+++); (C) strip cup test showing normal milk; (D) strip cup test showing milk of a animal undergoing clinical mastitis with several flocs (arrows)

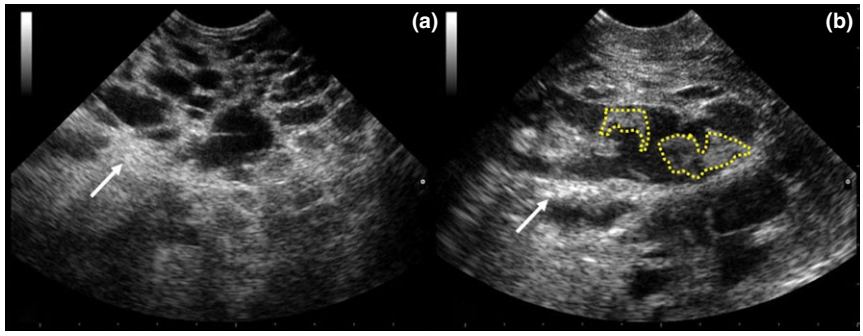

Fig. 2. Sonograms of goats' udder. (A) Health animal's sonogram. (B) Sonogram of a goat undergoing clinical mastitis, showing hyperechogenicity of the udder parenchyma when compared to the udder of a healthy animal (arrows) and the presence of flocs (echogenic structures) on the udder's cistern (highlighted area)

mixing with the reagent. The reaction occurs between the reagent and the genetic material of somatic cells in the milk, forming a gel which concentration is proportional to the number of somatic cells (Schalm and Noorlander, 1957). California Mastitis Test is a test originally developed to estimate the amount of somatic cells, which is indicative of inflammation, in bovine milk. Goat milk has physiologically more somatic cells than bovine's; test interpretation must take this account. The criteria used to diagnose subclinical mastitis were the ones cited by Contreras (1996) who recommend that subclinical mastitis can only be diagnosed with CMT in goats, when presenting stronger reactions of two and three crosses.

Concerning to sonographic evaluations, our observations are similar to those reported by Melo et al. (2012) in healthy goats (HG) and Rambabu et al. (2009) in non-lactating buffalo cows. Stocker and Rüsch (1997) reported similar sonographic characteristics of the udder in healthy cows and proposed that the degree of echogenicity depends on the milk content of the udder. Ultrasonography does not make possible to diagnose subclinical mastitis in dairy goats, as no abnormalities were observed on goats from SMG group when compared to HG.

The hyperechogenicity of the mammary parenchyma and presence of flocs observed are similar to those reported by Rambabu et al. (2009) in buffalo cows undergoing clinical mastitis and Fasulkov et al. (2014) in Bulgarian native goats. Hyperechogenicity can be explained by the inflammation process that results in tissue fibrosis, which has higher density than normal parenchyma.

As inflammation causes changes in echogenicity of affected organs (Feliciano et al. 2013), quantitative evaluation could be used to assess inflammation by attributing a numerical value to echogenicity. This novel, to the best of the authors' knowledge, set of observations may pave the way to the development of ultrasonography as a diagnostic tool in the clinical evaluations of the mammary gland in goats.

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Table 1. Quantitative echotextural attributes (mean ± SD) of the internal udder and teat structures in lactating goats

	Experimental groups			
	HG	SMG	CMG	p-value
Mammary parenchyma				
NPV	79.18 ± 24.11	80.55 ± 30.63	91.24 ± 27.46	0.59
Pixel SD	38.15 ± 9.43	31.67 ± 6.2	35.19 ± 7.98	0.21
Pixel min	0.95 ± 1.95	4.8 ± 6.26	3.27 ± 5.1	0.21
Pixel max	224.4 ± 33.52	207.9 ± 34.26	220.9 ± 33.77	0.52
Alveoli				
NPV	20.85^{a} ± 9.04	33.15^{ab} ± 24.29	50.12^{b} ± 10.31	0.005*
Pixel SD	20.08 ± 3.34	27.79 ± 18.51	25.65 ± 7.58	0.41
Pixel min	0.17 ± 0.19	4.86 ± 10.03	5.09 ± 3.9	0.23
Pixel max	149.4 ± 20.68	140.3 ± 51.27	155.6 ± 42.73	0.72
Gland cistern				
NPV	26.59^{a} ± 19.03	29.85^{a} ± 15.12	54.26^{b} ± 20.94	0.006*
Pixel SD	19.57 ± 9.03	24.63 ± 6.22	26.16 ± 6.58	0.15
Pixel min†	0 ± 0	0.1 ± 0.31	0.1 ± 0.33	0.6
Pixel max	183.7 ± 34.10	204.2 ± 40.5	211.1 ± 34.56	0.27
Teat cistern				
NPV	10.92^{a} ± 5.45	24.6^{ab} ± 13.79	30.52^{b} ± 21.27	0.02*
Pixel SD	13.39^{a} ± 4.26	22.41^{b} ± 6.17	21.16^{b} ± 7.62	0.005*
Pixel min†	0 ± 0	0 ± 0	0.22 ± 0.44	0.09
Pixel max	124.1^{a} ± 33.90	173.7^{b} ± 32.43	142.6^{ab} ± 45.67	0.02*

CMG: goats with clinical mastitis; NPVs: numerical pixel values or pixel intensity (0–255); Pixel SD: standard deviation of mean pixel values or pixel heterogeneity; Pixel MIN: minimum pixel intensity; Pixel MAX: maximum pixel intensity; HG, healthy goats; SMG, goats with subclinical mastitis.
Mean values followed by different superscript letters in the same line differ statistically by Tukey's test ($p < 0.05$).
*Significative for $p < 0.05$.
†Analysis between lines made by Kruskal–Wallis test ($p < 0.05$).

Table 2. Mean values for the echobiometry of health, undergoing subclinical and clinical mastitis goats' udder structures

	Experimental groups			
	HG	SMG	CMG	p-value
Udder's cistern diameter (cm)	45.14 ± 11.05	43.70 ± 7.82	37.43 ± 9.9	0.23
Udder's cistern area (cm^2)	11.49 ± 3.1	11.15 ± 6.12	8.32 ± 4.27	0.36
Teat cistern diameter (cm)	22.73 ± 11.82	27.25 ± 7.52	18.96 ± 11.35	0.25
Teat cisterna área (cm^2)	7.88 ± 3.65	9.64 ± 4.37	10.95 ± 10.06	0.63
Teat wall thickness	5.33 ± 1.49	3.83 ± 1.23	4.65 ± 1.4	0.07

CMG, goats with clinical mastitis; HG, healthy goats; SMG, goats with subclinical mastitis.
Tukey's test for means ($p < 0.05$).

Table 3. Mean values for the vascular indexes of the mammary artery in healthy, undergoing subclinical and clinical mastitis goats

	Experimental groups			
	HG	SMG	CMG	p-value
SPV (m/s)	0.19 ± 0.09	0.16 ± 0.71	0.21 ± 0.05	0.56
EDV (m/s)	0.19^{a} ± 0.07	0.14^{ab} ± 0.05	0.08^{b} ± 0.03	0.008*
RI	0.34 ± 0.23	0.48 ± 0.14	0.58 ± 0.08	0.06
PI	0.41^{a} ± 0.38	0.76^{ab} ± 0.46	1^{b} ± 0.26	0.03*

CMG, goats with clinical mastitis; SPV, peak systolic velocity; EDV, end diastolic velocity; RI, resistance index; PI, pulsatility index; HG, healthy goats; SMG, goats with subclinical mastitis.
Means followed by different superscript letters in the same line differ statistically on Tukey's test.
*Significative for $p < 0.05$.

Although Fasulkov et al. (2014) reported thickened teat wall on goats undergoing mastitis, it was not observed in our study. It could be explained by differences in methodology. The results obtained for udder's biometry from HG group are similar to those obtained by Slósarz et al. (2010) on the teats of goats before milking. Flöck and Winter (2006) examined ultrasonographically 52 cows diagnosed with diseases of the udder, between 1 and 14 days of lactation, and concluded that grey-scale ultrasonography could not replace bacteriological examination in a clinical setting but provides valuable additional information on the status of the udder that may consequently aid in prognosis. In our study, ultrasonography alone was capable of diagnosing clinical mastitis; however, we agree to the authors in what concerns to subclinical mastitis.

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Hemodynamic changes are part of the organism's 'attempt' to locally increase blood flow into the pathologically changed organs and tissues. Miranda and Domingues (2010) have reported that an increase in SPV values is also associated with increased blood flow in the diseased organs. Despite the fact that there was no significant statistical difference between the studied groups, animals of the CMG group presented higher SPV. The higher SPV can be explained by the necessity of the organism to make more defence cells get to the local of inflammation by increasing blood flow in the site of bacteria invasion. The differences found in PI could be explained by the same reason. Results obtained in this study can be considered a set of reference points and ranges to standardize computerized image analysis of the mammary gland ultrasonograms and Doppler values for the mammary artery in the diagnosis and monitoring of an array of pathological conditions (i.e. mastitis).

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

Teixeira PPM and Coutinho LN designed the experiment and standardized the ultrasonography technique with Feliciano MAR. Simplício KMMG and Sanchez DCC performed milk analysis. Barros FFPC and Almeida VT helped in the overall execution of the experiment. Bartlewski PM performed the pixels analysis of the sonograms and helped with Doppler methodology. Santos VJC performed the sonography examinations and wrote the article. Rodrigues LFS, Oliveira MEF and Vicente WRR revised it critically. All authors have approved the final version of the manuscript.

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