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Original Article

Ultrasonographic evaluation of the supramammary lymph nodes and udder's tissue in Saanen goat and its relation with subclinical mastitis

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Abstract

Background: Precise and on-time diagnosis of the udder's diseases is important, because of their economic importance. Udder structures like teat, parenchyma, and supramammary lymph nodes can be evaluated by ultrasonography. **Aims:** The study aimed to evaluate the ultrasonographic technique for imaging the supramammary lymph nodes and udder's tissue in Saanen goats and the relation between the findings of ultrasonography and subclinical mastitis. **Methods:** Thirty milking Saanen goats were evaluated in the study. Milk sampling from each teat was performed under standard conditions for bacteriological culture and somatic cell count (SCC). A 7.5 MHz linear transducer was used for the ultrasonography of teats with the water bath technique, and supramammary lymph nodes and udder's tissues were imaged using a 10 MHz linear transducer with direct contact. The length, height, area, and echogenicity of each lymph node and the teat canal wall diameter were measured using ImageJ 1.47v on the ultrasonography scanned images and analyzed by SPSS software. **Results:** There was no significant relationship between the dimension of the supramammary lymph nodes and SCC or culture. Age had a positive relationship with lymph node size. No significant relationship was seen between the size of the supramammary lymph node before and after the treatment. Supramammary lymph nodes' echogenicity of the quarter with subclinical mastitis and healthy ones represented no significant difference before and after the treatment. **Conclusion:** Ultrasonography of the udder, teat, mammary gland, and supramammary lymph nodes is a safe and non-invasive method for visualizing separate structures. The positive relationship between SCC and milk echogenicity as well as supramammary lymph nodes dimension, and age was described.

Key words: Subclinical mastitis, Supramammary lymph nodes, Ultrasonography, Saanen goat

Introduction

Mastitis is an inflammation of the mammary gland (udder) that causes a chemical and physical reaction in milk produced by goats. Dairy and meat goats are raised under intensive and semi-intensive management practices frequently. Depending on the severity of the disease, mastitis could result in decreased revenues for producers (LeiteBrowning, 2008).

Subclinical mastitis should always be suspected as one of the primary causes of decreased milk production in dairy goat flocks. The annual incidence of clinical mastitis in small ruminants is generally lower than 5%, but this incidence can increase sporadically. The prevalence of subclinical mastitis has been estimated at 5-30% or even higher. However, there is limited data about the incidence of goat and sheep intramammary infection (IMI) in the literature (Contreras *et al*., 2007; EZ Kotb *et al*., 2020).

Subclinical mastitis is defined as mammary gland inflammation without any visible clinical signs, often characterized by persistency. Detecting animals with subclinical mastitis requires additional diagnostic tests, such as individual SCC, California mastitis test (CMT), and bacteriologic culture (Menzies and Ramanoon, 2001). However, cut-off points of diagnostic tests in sheep and goats need to be defined as clearly as those in dairy cattle (Menzies and Ramanoon, 2001).

Somatic cell counts (SCC) are a commonly used indicator of udder health in cow, sheep, and goat milk (Constable *et al*., 2016). For dairy goats, the determination of the SCC and the interpretation of these values may be a problem. Several investigations have shown that even from not infected mammary halves,

SCC for goat's milk is often higher than that of cow's milk. Further, the predictive value of SCC as an indicator of the presence of IMI is obscured by its strong relationship with many other factors, such as lactation stage and milk yield (Menzies and Ramanoon, 2001).

Diagnostic ultrasonography is commonly used in human medicine to examine mammary glands alongside mammograms (Gooding *et al*., 2010). Ultrasonography helps describe the normal morphological appearance of sheep udder and teats (Rovai *et al*., 2008; Barbagianni *et al*., 2017). Furthermore, it is diagnostic for detecting and monitoring the pathological changes in the teats (Mavrogianni *et al*., 2004) and mammary glands (Flöck and Winter, 2006). Additionally, in cattle, ultrasonography can be used for different structures identified in udder parenchymas, such as hematoma and abscess (Lazaridis *et al*., 2012); ultrasonography can also be used to diagnose various pathological situations in caprine's udder (Fasulkov *et al*., 2014). It has been suggested that dimensions of the supramammary lymph nodes changes in some mastitis cases, and ultrasonography of the supramammary lymph node is probably a helpful method for confirmation of mastitis cases (Khoramian *et al*., 2015).

Few studies have reported the diagnostic application of udder ultrasonography in dairy goats (Fasulkov and Koleva, 2011; Fasulkov *et al*., 2013; Fasulkov *et al*., 2014). The present study's objective was to evaluate the relationship between the ultrasonography results of supramammary lymphatic glands, udder parenchyma, and teats, and intramammary infection in Saanen does with subclinical mastitis using B-mode ultrasonography technique (for possibility and repeatability).

Materials and Methods

In the current study, 30 Saanen does (normal or subclinical mastitis) were recruited at the farm of the Animal Science Faculty at Ferdowsi University of Mashhad. The age and weight of animals were in the range of 1-4 years and 23-53 kg, respectively. They were 60 to 150 days post-kidding. The goats were machine milked twice daily, post-teat dipping was used, and they were kept in separate roofed barns with a concrete surface for each goat. They were fed commercial hay and pellets, and drinking water was given *ad libitum*. Goats were allocated into two groups including healthy and subclinical mastitis. Milk bacteriologic culture was used as a standard method for detecting mastitis. Each goat with a positive bacteriologic culture was considered a "mastitic goat". "No growth" was considered as "free of mastitis".

Milk sampling

After removing contamination, pre-milking disinfection was performed using Bazal conium chloride spray. After 30 s, each teat was wiped with single-use paper separately. Teat ends scrubbed with an alcohol cotton pad. Three milking streams were discarded, and about 8 ml of milk was collected into the sterilized conical tube and sent to the laboratory of mastitis at the veterinary hospital, Ferdowsi University of Mashhad, kept at refrigerator temperature until use.

Ultrasonography examination

One skilled operator blindly performed the ultrasonography of supramammary lymph nodes, teat, and mammary parenchyma. Additionally, ultrasonography was repeated one month after treatment in a group of goats with a positive bacteriologic culture of milk samples.

A portable ultrasound machine (Mindray®, DP6600 vet) with a 7.5-10 MHz linear transducer (contact area 6 × 1.5 cm) was used, and parameters were measured on saved images with ImageJ 1.47v software.

Ultrasonography of teat

Goats were kept in a standing position covering their eyes to prevent movement. Teats were scanned longitudinally with a 10 MHz linear transducer using the water bath technique. The diameter of both teat walls dorsal to the teat canal (the border between the teat and gland cistern) was measured at least three times for more accuracy, and the heist diameter was considered the teat wall diameter (Fig. 1).

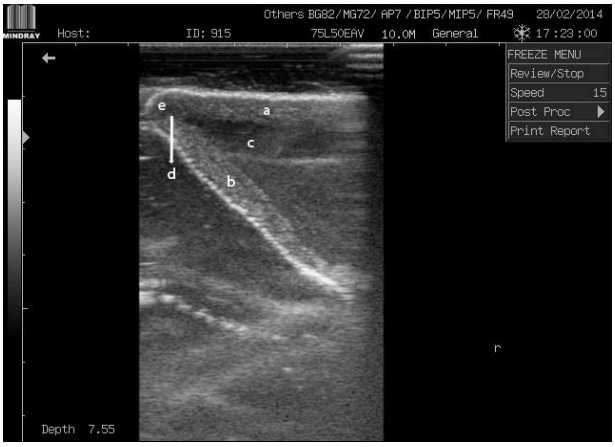

Fig. 1: Ultrasounds scan of the teat (longitudinal plane) via water bath technique in Saanen goat. a: Lateral teat wall, b: Medial teat wall, c: Teat cistern, d: Furstenberg's rosette, and e: Teat canal

Ultrasonography of glandular parenchyma

After setting animals in a recumbent position on the pad, forelegs, and hind legs were restrained. After shaving and degreasing the skin with alcohol and applying coupling gel, a 7.5 MHz linear transducer was placed lateral and caudal directly onto the udder skin to examine the parenchyma. The Udder parenchyma surface was divided into proximal, middle, and distal regions, and each region was scanned separately, and any abnormality was recorded. A quantitative assessment of milk in ducts and cistern was performed, and the echogenicity of the images was scaled 0 to 3; scaling was performed based on the percentage of floating echogenic particles to total area (<25%, 25-50%, 50-75%, and >75% indicated scale 0 to 3, respectively).

Ultrasonography of supramammary lymph node

For taking images with better quality, the hair of the area was clipped at both sides. Ultrasonography examination was performed in a longitudinal plane without transducer pressure on the skin. The length (dorsoventral dimension), depth (caudocranial dimension), and area of each lymph node at both halves were measured separately by ImageJ 1.47v software on saved images for quantifying echogenicity of lymph node, and average values of histogram were determined via ImageJ software. It was repeated at least three times on each lymph node for more accuracy (Fig. 2).

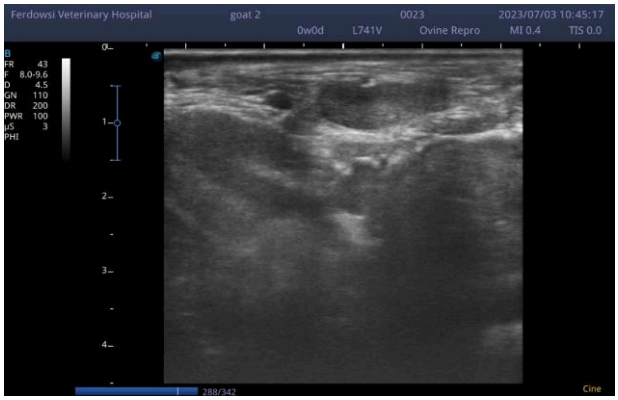

Fig. 2: Longitudinal section of the ultrasound image of a supramammary lymph node in a Saanen goat

Bacteriological procedures

Milk samples were collected from both udder halves to perform a bacteriological culture. 0.01 ml milk sample was spread onto a blood agar plate containing 5% of sheep red blood cells and a MacConkey agar plate. All plates were incubated at 37°C and examined for bacteriologic growth after 24 h.

Colonies grown on each plate were examined by gram stain; if gram-positive cocci were seen, the colony was cultured again on a blood agar plate. Catalase tests were performed to differentiate *Staphylococcus* and *Streptococcus* species. For identifying *Streptococcus* species, esculin agar media, CAMP, and hippurate hydrolysis tests were carried out. The tests were conducted to identify *Staphylococcus* species including the culture of mannitol salt agar, DNase media, and VP (Voges Proskauer).

If gram-positive coccobacilli were seen, due to the probability of *Corynebacterium* spp., catalase test and morphologic appearance of colonies were considered. White to grey and dry colonies without hemolysis on blood agar plates are typical of *Corynebacterium* species.

MacConkey agar plates were examined after the incubation period. Lactose-positive (pink-red appearance) and lactose-negative (white-colorless appearance) colonies were chosen for further investigations. TSI, Simmons citrate, and motility medium were used to detect unknown colonies.

Somatic cell count (SCC)

Milk samples transferred to the laboratory within 24 h were evaluated with a 5000 somatic device (Denmark) to count the somatic cells. If sample transferring took more than 24 h, potassium dichromate (8 mg/ml milk) was added to milk samples to prevent any changes in SCC.

Treatment

Treatment of udder halves with positive bacteriologic culture was performed with the intramammary infusion of cefquinome (Cobacter®, Kimia Biotech, Iran) and systemic administration of 20% tylosin (Tyloject®, Aburaihan, Iran) for three days. Milk sampling and ultrasonography were repeated on day 10 after treatment.

Statistical analysis

The relationship of dimensions (length, depth, and area) of lymph nodes and teat wall thickness and intra-mammary infection were analyzed by linear regression test. Age and weight were included as covariants in all of the regression tests. A backward stepwise procedure was used for independent variables with a significant relationship ($P<0.05$) with length, height, area of lymph nodes, teat wall thickness, and SCC.

Regarding the normality of data, pair sample tests were used to compare SCC, length, height, and lymph nodes area before and after treatment. Considering the normality of data, the Mann-Whitney U test was used to compare lymph nodes, and cisterns echogenicity between left and right udder halves. Mann-Whitney U test was also used to compare the echogenicity of lymph nodes and cistern between infected and healthy udder halves. The relationship between the echogenicity of lymph nodes and cisterns with SCC was analyzed with the Spearman correlation test. A Wilcoxon signed-rank test was used to compare the echogenicity of lymph nodes and cisterns before and after treatment. All data were analyzed using SPSS package version 19, and $P\leq0.05$ was considered significant.

Results

60 udder halves from 30 goats were included in this study; 16.66% (n=10) of them were positive for bacteriologic culture. Between positive cases, 3 *Staphylococcus aureus* (30%), 3 *Streptococcus uberis* (30%), 2 *Bacillus* spp. (20%), 1 *E. coli* (10%), and 1 coagulase-negative *Staphylococci* (CNS) (10%) were isolated. There was no contaminated milk sample in the present study.

Eight infected halves had been cured after treatment, and two udder halves were still positive. From those two infected halves, *S. aureus* and *S. uberis* were isolated.

Ultrasonography of glandular parenchyma

A linear transducer (7.5 MHz) with direct contact was used for the ultrasonography of parenchyma. Abnormal changes like calcification and increased echogenicity of the affected teats were not detected.

Ultrasonography of supramammary lymph nodes

The superficial supramammary lymph nodes were located at the upper part of the mammary gland and were quickly found by palpation. They were usually well demarcated from the surrounding tissue, represented as anechoic structures, oval, with a thin echogenic capsule surrounding them. All 'normal' nodes had a well-defined echogenic linear structure through the node's center but were not seen ultrasonographically in all cases. In five goats, one small lymph node was detected in addition to the primary lymph nodes (Fig. 2).

SCC, length, height, and area of lymph nodes in left and right teat halves

The mean of SCC in normal goats for left and right udder halves was 834.5×10^3 cells/ml and 1003×10^3 cells/ml, respectively. The average length, height, and area of lymph nodes in the left udder half were 22.60 ± 4.6 mm, 10.52 ± 2.55 mm, and 195.91 ± 77.67 mm^2, and in the right udder, half were 22.05 ± 6.63 mm, 10.16 ± 1.91 mm, and 197.40 ± 106.11 mm^2, respectively (Table1).

The comparison of SCC, length, height, and area of lymph nodes in normal goats and goats with subclinical mastitis

The mean of SCC in infected and healthy udder halves was 1391.13×10^3 cells/ml and 862.05×10^3 cells/ml, respectively. The average length, height, and area of lymph nodes for infected udder halves were 25.11 ± 10.85 mm, 10.42 ± 2.09 mm, and 246.76 ± 173.47 mm^2, respectively. For healthy udder halves, 21.58 ± 4.38 mm, 10.33 ± 2.28 mm, and 188.95 ± 72.45 mm^2 were measured for length, height, and area of lymph nodes, respectively (Table 2). Dimensions of lymph nodes did not demonstrate any significant relationship with intramammary infection, weight, and SCC.

The effect of age on length, height, and area of lymph node

Length, height, and area of lymph nodes had a significant linear relationship with age; one year increase in age of goats was accompanied by 2.57 mm, 0.91 mm, and 40.55 mm^2 increase in length, height, and area of lymph nodes, respectively (Table 3).

Table 1: SCC, length, height, and area of lymph nodes in left and right teat halves individually in Saanen goats

Teat half	Descriptive statistics	SCC $\times 10^3$ (cell/ml)	Length (mm)	Height (mm)	Area (mm^2)
Left	Number	28	30	30	30
	Mean	834.5	22.05	10.52	195.91
	Standard deviation	726.97	4.6	2.55	77.67
	Minimum	31	14.33	5.26	76.12
	Maximum	2858	34.76	17.73	414.24
Right	Number	28	30	30	30
	Mean	1003	22.05	10.16	197.4
	Standard deviation	1094.58	6.63	1.91	106.11
	Minimum	96	13.84	6	61.08
	Maximum	4265	51.08	13.01	664.7
Total	Number	56	60	60	60
	Mean	918.72	22.05	10.34	196.66
	Standard deviation	924.57	5.66	2.24	921.95
	Minimum	31	13.84	5.26	61.08
	Maximum	4265	51.08	17.73	664.7

Table 2: SCC, length, height, and area of lymph nodes in normal goats and goats with intramammary infection in Saanen goats

Mastitis	Descriptive statistics	SCC $\times 10^3$ (cell/ml)	Length (mm)	Height (mm)	Area (mm^2)
Healthy	Number	50	52	52	52
	Mean	862.05	21.58	10.33	188.95
	Standard deviation	844.51	4.38	2.28	72.45
	Minimum	31	13.84	5.26	61.08
	Maximum	4141	34.76	17.73	414.24
Infected	Number	6	8	8	8
	Mean	1391.13	25.11	10.42	246.76
	Standard deviation	1453.81	10.85	2.09	173.47
	Minimum	405	17.37	7.57	108.68
	Maximum	4265	51.08	13.01	664.7
Total	Number	56	60	60	60
	Mean	918.76	22.05	10.34	196.66
	Standard deviation	924.57	5.66	2.24	921.95
	Minimum	31	13.84	5.26	61.08
	Maximum	4265	51.08	17.73	664.7

No relationship existed between SCC, intramammary infection, age, and weight.

Relationship between teat wall thickness and intramammary infection, age and weight

In the present study, the water bath technique was used for the ultrasonography scan of the teat because of the high-quality images compared to the usual technique. For taking images with higher resolution, the teat and gland cistern must be contained milk.

Results showed no significant relationship between teat wall thickness and other variables such as intramammary infection, age, and weight. The mean, minimum, maximum, and standard deviations of teat wall thickness are shown in Table 4.

The comparison of SCC and length, height, and area of lymph nodes before and after treatment

The mean, maximum, minimum, and standard deviation of SCC and length, height, and area of lymph nodes before and after treatment are shown in Table 5. SCC before and after treatment did not significantly change (P=0.07). Also, the length (P=0.372), height (P=0.167), and area (P=0.282) of lymph nodes did not demonstrate any significant differences before and after treatment.

The echogenicity of lymph nodes, gland cistern, and SCC

There was a significant relationship between the echogenicity of gland cistern and SCC, as an increase in SCC was associated with an increase in milk cistern echogenicity (P=0.001, r=0.43).

The median, first quartile, third quartile, maximum and minimum of SCC, echogenicity (Histogram Analysis) of lymph nodes, and milk cistern for right and left halves were mentioned in Table 6.

There was no significant relationship between lymph nodes and milk cistern of the right and left halves regarding echogenicity (P=0.210, P=0.524).

Relationship between echogenicity of lymph node and milk cistern and subclinical mastitis

The median, first quartile, third quartile, maximum and minimum of SCC, echogenicity (Histogram Analysis) of lymph node, and milk cistern for healthy and infected goats are shown in Table 7.

There was no significant relationship between lymph nodes and milk cistern echogenicity in healthy and infected udder halves (P=0.520, P=0.894).

Table 3: Length, height, area of lymph node, and age in Saanen goat

Dimensions	Age	β	Standard error	Significant level	Upper level	Lower level	R^2
Length (mm)	Constant	14.76	2.15				0.185
	Age	2.57	0.72	0.001	1.13	4.02	
Height (mm)	Constant	7.74	0.87				0.147
	Age	0.91	0.29	0.003	0.32	1.49	
Area (mm^2)	Constant	81.47	35.38				0.173
	Age	40.55	11.86	0.001	16.79	64.32	

β: Regression coefficient, and R^2: Correlation coefficient

Table 4: Teat wall thickness of the left and right teat halves and intramammary infection in Saanen goat

Variables	Teat	Number	Mean (mm)	Standard deviation (mm)	Minimum (mm)	Maximum (mm)
Teat half	Left	29	5.78	1.28	3.93	9.21
	Right	30	5.68	1.64	3.53	9.67
Mastitis	Healthy	51	5.61	1.31	3.53	8.95
	Infected	8	6.46	2.13	3.70	9.67
	Total	59	5.72	1.46	3.53	9.67

Table 5: SCC and Length, height, and area of lymph node before and after treatment in Saanen goat

Variables	Treatment	Number	Mean	Standard deviation	Minimum	Maximum
SCC $\times 10^3$ (cell/ml)	Pre-treatment	6	883.17	397.79	405	1452
	Post-treatment	6	4731.8	4146.97	445	9180
Length (mm)	Pre-treatment	6	25.61	12.73	17.37	51.08
	Post-treatment	7	21.47	4.17	17.59	29.91
Height (mm)	Pre-treatment	6	10.97	2.05	7.57	13.01
	Post-treatment	7	9.97	1.76	6.88	12.44
Area (mm^2)	Pre-treatment	6	259.07	202.8	108.68	664.7
	Post-treatment	7	186.1	65.68	110.72	316.93

Table 6: Echogenicity of lymph node, gland cistern and SCC in Saanen goat

Variables	Teat half	Minimum	First quartile	Median	Third quartile	Maximum
SCC $\times 10^3$ (cell/ml)	Right	96	328.5	596	1334	4265
	Left	31	205.25	611	1232	2858
Histogram Analysis of the lymph node	Right	31.25	35.91	40.06	51.7	59.91
	Left	30.89	39.22	46.85	56.16	89.55
Cistern echogenicity (grade 0-3)	Right	0	0	0	0.5	2
	Left	0	0	0	0	2

Table 7: Echogenicity of lymph node, gland cistern and SCC in Saanen goat

Variables	Subclinical mastitis	Minimum	First quartile	Median	Third quartile	Maximum
SCC $\times 10^3$ (cell/ml)	Healthy	31	211.75	519	1200	4141
	Infected	405	596	950	1452	4265
Histogram Analysis of the lymph node	Healthy	30.89	37.64	43.56	51.55	89.55
	Infected	29.8	30.3	51.65	56.83	73.99
Cistern echogenicity (grade 0-3)	Healthy	0	0	0	0.25	2
	Infected	0	0	0	0	1

Table 8: SCC, echogenicity of lymph nodes and milk cistern before and after treatment in Saanen goat

Variables	Treatment	Minimum	First quartile	Median	Third quartile	Maximum
SCC $\times 10^3$ (cell/ml)	Pre-treatment	405	596	950	1452	4265
	Post-treatment	445	847	4739	8492	9180
Histogram Analysis of the lymph node	Pre-treatment	29.8	30.3	51.65	56.83	73.99
	Post-treatment	42.49	43.44	49.25	55.23	75.77
Cistern echogenicity (grade 0-3)	Pre-treatment	0	0	0	0	1
	Post-treatment	0	0	0.5	2	3

SCC, echogenicity of lymph node and milk cistern before and after treatment

Median, first quartile, third quartile, maximum and minimum of SCC, and echogenicity (Histogram Analysis) of lymph nodes and milk cistern in infected udder halves before and after treatment are shown in Table 8.

There was no significant relationship between lymph nodes and milk cistern echogenicity of halves before and after treatment (P=0.721, P=0.081).

Discussion

In the present study, the water bath technique was used for the ultrasonographic examination of the teats in the goats. Studies that performed the same technique on cattle (Stocker and Rüsch, 1997; Dinç *et al*., 2000; Santos *et al*., 2004; Franz *et al*., 2009; Fasulkov, 2012), and goats (Fasulkov *et al*., 2010; Fasulkov and Koleva, 2011; Fasulkov *et al*., 2013; Fasulkov *et al*., 2014) mentioned that this technique had the advantage of high-quality images comparing to others. Less pressure in this technique leads the structures of the teat can be appropriately shown on the image with appropriate quality. The technique also enables the examiner to keep a hand free to move the teat into position and to handle the machine. In contrast, the other hand holds the cup of water and moves the probe simultaneously in a vertical plane (Franz *et al*., 2009).

For better differentiation of the teat canal on the image, the teat and gland cistern needed to contain milk, and milking must be done immediately before scanning. Dinc and Sendag (2000) reported that scanning of teat full of milk resulted in better-quality images. Franz *et al*. (2001) confirmed that filling the teat in lactating cows with sterile water or normal saline solution does not seem to be necessary. The milk in the teat canal prevents the overlying of walls on each other and makes the walls and canal be seen separately on the ultrasound image.

This study used a 10 MHz linear transducer to scan the teat to get a high-quality image. In contrast, most authors recommended using a linear probe with a frequency of more than 7.5 MHz (Franz *et al*., 2001; Couture and Mulon, 2005; Flöck and Winter, 2006; Fasulkov and Koleva, 2011). However, Fasulkov and Koleva (2011) indicated that a 10 MHz probe provided a high-quality image.

The transducer was placed directly onto the udder skin for better imaging of the parenchyma. Other researchers have also used the direct contact technique (Fasulkov *et al*., 2010; Fasulkov and Koleva, 2011; Fasulkov *et al*., 2013; Fasulkov *et al*., 2014).

In normal conditions, the supramammary lymph node was a hypoechoic structure, well demarcated from the surrounding tissues, with an echogenic linear structure through the node's center; however, it was not readily visible in all cases (about 50%). Bradley *et al*. (2001) described supramammary lymph nodes in cattle and

mentioned that the parenchyma of the node ranged from almost anechoic in some cases to hypoechoic with more hyperechoic 'speckles'. In those nodes, a well-defined echogenic linear structure is depicted longitudinally through the center, with a thin echogenic capsule surrounding it. Bradley *et al*. (2001) motioned that some cows had a smaller and deeper node which was always smaller and less well-defined than the main one (the superficial lymph node) and its ultrasonographic features were similar.

The mean dimensions (length, height, and area) of lymph nodes did not demonstrate a significant relationship with the occurrence of mastitis, weight, and SCC. However, they had a significant relationship with the age of goats. In another study, no correlations existed between the lymph node parameters and SCC. However, there was a positive correlation between the lactation number and all the lymph node parameters (Bradley *et al*., 2001). This difference may be because the authors measured SCC in a composite milk sample, representing the four quarters, including healthy ones, causing a reduction of SCC. In two trials conducted on dairy cattle, CMT-positive cows had larger supramammary lymph nodes (Bradley *et al*., 2001; Khoramian *et al*., 2015). Khoramian *et al*. (2015) indicated that in the cows, one or two-quarters of each side was positive in bacteriological culture. The length and depth of ipsilateral nodes were significantly larger than sides with two-quarters with negative culture. Risvanli *et al*. (2019) represented that in a cow with clinical mastitis, there was a significant difference between the groups (normal and clinical mastitis) in the terms of length, width, circumference, and cortex thickness as well as echogenicity, calcification, and border irregularity of the supramammary lymph node. The threshold of 1×10^6 cells/ml reported by Poutrel and Lerondelle (1983) will identify most infected halves, but uninfected halves considered as infected is excellent (about 30%). Lerondelle *et al*. (1992) showed that the most discriminating threshold for diagnosis was a cell count of $0.8 \times l0^6$ cells/ml. Dairy factories in Poland use the threshold of 2×10^6 of the SCC in goat milk. However, Bagnicka *et al*. (2011) showed that the bacterial pathogens were present in about 20% of milk samples containing low SCC (below 1×10^6/ml). Therefore, the SCC cannot be the only decisive indicator of bacterial infection of the mammary gland in goats. In the present study, lymph node dimensions of infected halves were slightly bigger than those of healthy teat halves but had no significant differences. The reason could be the low number of subclinical mastitis cases and the time interval from initiating infection.

The size of supramammary lymph nodes in cattle is very variable and is related to the lactation number, mastitis history, and individual quarter cell counts (or CMT) of the cows; as a result, the size range for a 'normal' lymph node is hard to define (Bradley *et al*., 2001).

Teat wall thickness had no significant relationship with the occurrence of mastitis, age, and weight. Ultrasound studies on the mammary gland in goats suffering from acute mastitis revealed that the image of the teat half in these animals exhibited a thickened hyperechoic teat wall (Fasulkov and Koleva, 2011). In research performed on the Egyptian Baladi goat, a thick and hyperechoic teat wall was noted in chronic mastitis. A lack of clear visualization of the teat canals was revealed in a goat with subclinical mastitis (EZ Kotb *et al*., 2020). In our study, differences were insignificant despite a slight tendency in the teat wall thickness of the infected gland. Factors such as small sample size, type and causing mastitis pathogen, and time interval from initiation of infection and ultrasound scanning might be involved in this insignificant relationship.

There were no significant differences for the SCC of milk before and after treatment (P=0.070); also, length (P=0.372), height (P=0.167), and area (P=0.282) of lymph nodes before and after treatment did not change significantly. Other studies did not indicate comparisons of SCC and lymph node dimensions before and after treatment. After treatment, lymph node dimensions decreased slightly but without significant differences. Factors such as small sample size, type and causing mastitis pathogen, and short interval between infection and scanning might be involved.

Using the spearman correlation test, our data showed that milk cistern echogenicity was correlated with SCC, so increasing SCC was associated with an increase in echogenicity of milk cistern (P=0.001, r=0.43). A correlation was noted between the increase in SCC and the number of echogenic particles in the milk cistern. Those particles were visible as echogenic floating structures in the milk cistern and subsequently enhanced echogenicity. This correlation was not reported in other studies.

Lymph node and milk cistern echogenicity did not differ between infected and healthy teat halves. This lack of difference in echogenicity of healthy and infected udder halves, which had not been reported in the literature, might be due to the interval between infection and scanning, small sample size and type, and causing the pathogen of subclinical mastitis.

Lymph nodes and milk cistern echogenicity were not different before and after treatment. This comparison should have been mentioned in the literature. Factors such as small sample size, type and causing pathogen of mastitis, and short interval from treatment to scanning might have been involved.

The echogenicity of the udder parenchyma was not changed significantly in the affected group in the current study. EZ Kotb *et al*. (2020) revealed non-homogenous and hypo to hyperechoic mammary gland parenchyma structure in a goat with subclinical mastitis.

In conclusion, ultrasonography of the udder and teat is a safe and non-invasive method suitable for visualizing separate structures of the mammary gland (teat and parenchyma) and supramammary lymph nodes in Saanen goats.

This study demonstrated that the size of supramammary lymph nodes became significantly larger

during the aging of goats. Moreover, the echogenicity of the milk cistern was significantly increased concerning the increase in somatic cell count.

This study could be a preliminary study for evaluating the ultrasonographic findings in the diagnosis of subclinical mastitis. Because of the low number of infected teat halves in the present study, further investigations need to be done on a more significant number of goats to establish the usefulness of ultrasonography as an evaluation tool for diagnosing mammary gland infection. Further studies are warranted, including examining supramammary lymph nodes in goats at the different stages of lactation and in different lactation periods.

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Conflict of interest

The authors declared no conflict of interest.

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