Ultrasound evaluation and diagnostic grading of mammary gland cistern in quarters of dairy cows affected by mastitis

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SUMMARY

Mastitis, the inflammation of the mammary gland, represents one of the main pathologies in dairy cattle farm. It leads to severe economic losses and drug administration, representing a relevant problem for animals, farmers, and public health. Diagnosis mainly consists in analysis on milk, such as microbial culture, PCR and somatic cell count. Considering the vitally importance of a fast and reliable diagnosis, the aim of this research is to evaluate ultrasound mammary examination as an on-farm tool able to timely detect and characterize quarters affected by mastitis. For this purpose, 39 primiparous and multiparous dairy cattle from a single farm in Veneto Region (Italy) have been enrolled. For each animal, clinical examination, ultrasound examination of the mammary gland and milk sampling of single quarter have been performed. Animals have been divided into four groups based on symptoms, microbial culture and somatic cell count. Specifically, CTR" (control, having no clinical signs, negative microbial culture and SCC< 200.000 cells/ml), "IMI" (Intramammary infection, having no clinical signs, positive microbial culture and SCC <200.000 cells/ml), "SUB" (subclinical, having no clinical signs, positive microbial culture and SCC ≥200.000 cells/ml) and "CL" (clinical, having clinical signs, positive microbial culture and SCC ≥200.000 cells/ml). An ultrasound classification of the echogenicity of the content of the mammary gland cistern has been proposed from 0 (completely anechoic cistern), to 3 (completely echogenic cistern). Considering the non-homogenous distribution of data, Kruskal-Wallis rank sum test has been applied. Results show significant differences between groups of SCC (means ± standard deviation 18.39±3.21, 33.75±5.12, 608.71±148.50, 1267.20±94.98 from group 0 to 3 respectively) and ultrasound score (means ± standard deviation 0.71±0.2, 0.84±0.11, 1.00±0.38, 2.60±0.24 from group CTR to CL respectively. Moreover, SCC values were significantly different also considering US Score (means ± standard deviation 32.52±7.25, 91.92±39.11, 109.08±57.78, 412.07±159.52 from score 0 to 3 respectively). Even though further studies are needed in order to validate and deepen this technique, these results suggest a possible application of ultrasound examination as a fast and immediate detection instrument of mastitis.

KEY WORDS

Dairy cattle; Diagnosis; Mammary ultrasonography; Mastitis; On-farm.

INTRODUCTION

Mastitis is an inflammation of the mammary gland in dairy cattle, primarily caused by bacterial infection. It remains one of the most common diseases in dairy farming, impacting animal health and farm economics. Major losses from mastitis include reduced milk production, discarded milk, increased veterinary costs, and increased culling rate. A 2019 study in Lombardy, Italy, found that 50% of dairy herds tested positive for contagious mastitis pathogens, which often require antimicrobial treatment. However, this contributes to concerns about antibiotic resistance, as many pathogens involved in mastitis can also affect humans.

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Mastitis can be classified by pathogen type-environmental or contagious-and by symptoms-clinical or subclinical. Environmental mastitis is caused by microorganisms in the environment, while contagious mastitis spreads from infected to healthy cows, often during milking. Clinical mastitis involves visible symptoms like milk changes, swollen udders, and systemic illness. Over 135 microorganisms are known to cause intramammary infections, with staphylococci, streptococci, and gram-negative bacteria being the most common.

Mastitis diagnosis typically involves microbial culture or PCR to identify the pathogen, allowing for targeted treatment and better management. Inflammatory responses can also be assessed using indicators such as somatic cell count (SCC), electrical conductivity, and specific enzyme levels. Although a raised SCC is a recognized indicator of a bacterial infection, a very low SCC has been associated with an increased susceptibility to clinical mastitis. Various cow-side methods have been developed and studied in order to measure or estimate milk SCC directly on farm, allowing for timely diagnosis. These include the California Mastitis Test (CMT) and Wisconsin Mastitis Test (WMT). Other on-farm tools include On-Farm Culture, Infrared Thermography and, more recently, ultrasound mammary evaluation. Ultrasound is a non-invasive technique that provides real-time tissue visualization through grayscale and a 2D imaging. It has been extensively used on cattle farms for various purposes, including gynecology, reproduction, body condition scoring, marbling evaluation, and diagnosing respiratory and metabolic conditions.

Its application to mammary gland evaluation is more recent. To capture images of the glandular parenchyma, the gland cistern (gland sinus), and the border between the gland cistern and the teat cistern (teat sinus), the transducer must be applied directly to the mammary gland. Physiologically, udder parenchyma appears as a homogeneous echoic structure with few anechoic vessels and milk ducts. Mammary gland cistern, instead, can be described as a large anechoic area, due to the abundant milk content. Some studies already applied this technique in order to predict productive strength of dairy cows, such as daily milk yield.

Ultrasonographic characteristics of mammary glands affected with mastitis have been described in cows, ewes, sows, and bitches. In dairy cows, teats and mammary glands parenchyma have been examined via ultrasonography in both healthy and diseased cases. Macroscopic alteration during clinical mastitis, such as abscesses, gas formation, haematoma, oedema have well been described in a study of 2004. Another article found the non-homogeneity of udder parenchyma in the first examination as a reliable factor able to predict outcome of clinical mastitis. A non-homogeneous hypoechoic pattern was imaged by Amorim in case of acute mastitis while a hyperechoic pattern was imaged in chronic mastitis as a result of fibrosis. A 2020 study described the udder parenchyma affected by subclinical mastitis as homogenous hypoechoic with lack of clarity of visualization of milk alveoli and lactiferous duct. Moreover, they highlighted a loss of anechogenicity of gland cisterns with hypoechogenic content, in-stead. Finding of echogenic solid content in gland cistern was in agreement with another article of 2015.

Considering the importance of a timely and accurate diagnosis of mastitis, the aim of this study is to assess the ultrasound evaluation of the mammary gland cistern as reliable diagnostic tools for the detection and characterization of mastitis.

MATERIAL AND METHODS

Animals' enrolment and experimental design

This study was carried out within Agritech National Research Center, and was approved by the Ethical Committee of the University of Padua (Prot. N. 20804/2024). Animal care and procedures were in accordance with the European directive 2010/63/EU and the national law D.L.2014/26.

For this study, 39 primiparous and multiparous dairy cattle have been enrolled. Specifically, 8 cattle were primiparous, 9 were at second lactation, 5 at third lactation, 5 at fourth lactation, 4 at fifth lactation, 3 at sixth lactation, 2 at seventh lactation, 1 at eight lactation, 2 at tenth lactation.

All animals have been clinically examined in order to preliminary detect clinical mastitis, with a specific focus on milk macroscopic alterations, possible mammary quarters inflammatory indicators and systemic signs. A control group represented by healthy animals (no systemic signs, SCC< 200.000 cells/ml and negative microbial culture) has been included. To avoid bias due to management or environmental factors, a single farm in Veneto region, Italy, having high incidence of mastitis caused by Coagulase Negative Staphylococci (CoNS) has been selected. Moreover, to reduce animals-related differences, only Simmental cows have been considered. Animals with other pathologies, differing from mastitis, or being treated in the same lactation with systemic or local antibiotics, have been excluded. Data regarding number of lactations and days in milk (DIM) have been collected. For each animal, sterile milk sampling of the single quarters has been performed and transported within 12h to Istituto Zooprofilattico delle Venezie (IZSVe) in order to perform microbial analysis and SCC. Then, 6 hours from the morning milking, ultrasound mammary evaluation of the single quarters have been performed.

Based on the result of the clinical examination and milk analysis (performed as described in the next sub-section), animals have been divided into 4 groups: "CTR" (control, having no clinical signs, negative microbial culture and SCC< 200.000 cells/ml), "IMI" (Intramammary infection, having no clinical signs, positive microbial culture and SCC <200.000 cells/ml), "SUB" (subclinical, having no clinical signs, positive microbial culture and SCC ≥200.000 cells/ml) and "CL" (clinical, having clinical signs, positive microbial culture and SCC ≥200.000 cells/ml).

Data collected have then been statistically analysed.

Milk sampling and laboratory analysis

Single quarter milk samples were collected aseptically from each quarter of each cow during the evening milking following National Mastitis Council (NMC) guidelines Briefly, the teats were previously cleaned, stripped, predipped and dried, then they were scrubbed using 70% alcohol and eventually 3 streams of milk were collected in a tube maintained with a 45° inclination, in order to avoid contamination. The samples were labelled and kept refrigerated at 4°C until the delivery to the IZSVe laboratory. Within 24 hours from the collection, the SCC and the milk culture were performed on each sample.

Briefly, 0.01 ml of milk was streaked on a portion of a blood agar plate, and incubated at 35 to 37°C overnight in a CO2 incubator. Plates were examined for growth at 24 and 48 h. Bacteria were identified by colony morphology and Gram stain. SCC was measured with a DeLaval cell counter (DCC; DeLaval International AB, Tumba, Sweden) according to the manufactor instructions, milk culture was performed according to the NMC guidelines . Pathogen's identification was confirmed using Matrix Assisted Laser Desorption Ionization - Time of Flight mass spectrometry (MALDI-TOF MS), with the MALDI Biotyper System (Bruker Daltonik GmbH, Bremen, Germany).

Considering that 3 cows had only 3 quarters in milking, due to acute mastitis process in previous lactations, a total of 151 mammary quarters have been sampled.

Ultrasound classification of mammary gland cistern

Mammary gland cistern, in case of no alteration, appear as a large homogeneous anechoic area.

According to this, 6 hours from morning milking, ultrasound mammary evaluation has been performed. For this purpose, a portable ultrasound scanner (Draminski Blue; Draminski® S.A., Olsztyn, Poland) equipped with a multi-frequency convex probe (B mode; Frequency 6.5 MHz; Depth 150 mm; Focus 50 mm) has been used. After been sprayed with alcohol 90% (as transducing agent), each quarter has been longitudinally scanned, with a specific focus to mammary gland cistern. The probe was placed on the upper lateral (front quarters) and caudal (hind quarters) surfaces, scanning the structures going downward toward the teat. Captures of mammary gland cistern have been registered in order to classify them. An Ultrasound Score (US Score) from 0 to 3 based on echogenicity, has been proposed in our study as explained hereafter and shown in table 1.

US Score 0 was assigned to completely anechoic mammary gland cistern aspect.

US Score 1 was assigned in case of presence of little echogenic spots in the gland cistern.

US Score 2 was assigned to mammary gland cistern presenting massive echogenic spots.

US Score 3 was assigned to completely echoic mammary gland cistern.

Statistical analysis

Statistical analysis was performed using the R version 4.3.2. Based on the clinical examination, SCC and microbiological analysis, animals have been divided into 4 groups. Before starting the analysis, Shapiro-Wilk test has been applied in order to evaluate normal distribution of data. Non-normal distribution of data has been assessed. From this statement, means and standard error of means (SEM) of SCC, DIM, number of lactations have been calculated. Then, Kruskal-Wallis rank sum test have been applied in order to evaluate significative differences between groups, and between different US Score. Significance has been considered with p-value≤0.05.

Table 1 - Visive explanation of criteria to assign Ultrasound Score to mammary gland cistern

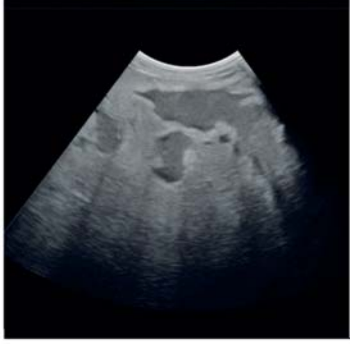
US Images	US Score
	US Score 0: completely anechoic mammary gland cistern (Dairy cattle; B mode; Frequency 6.5 MHz; Depth 150 mm; Focus 50 mm)
	US Score 1: small echogenic spots (Dairy cattle; B mode; Frequency 6.5 MHz; Depth 150 mm; Focus 50 mm)
	US Score 2: massive echogenic spots (Dairy cattle; B mode; Frequency 6.5 MHz; Depth 150 mm; Focus 50 mm)
	US Score 3: completely echogenic mammary gland cistern (Dairy cattle; B mode; Frequency 6.5 MHz; Depth 150 mm; Focus 50 mm)

Table 2 - Means and Standard Error of Means (SEM) of Days in Milk (DIM, number of lactations, somatic cell count (SCC) and Ultrasound Score (US Score) per Group.

Variables	CTR (means±SEM)	IMI (means±SEM)	SUB (means±SEM)	CL (means±SEM)
DIM (days)	242.06±11.41	270.71±10.98	209.86±45.23	179.40±36.55
Number of lactations	3.61±0.31	3.43±0.28	4.29±0.71	4.00±0.89
SCC x 10^3 (cells/ml)	18.39±3.21	33.75±5.12	608.71±148.50	1267.20±94.98
US Score	0.71±0.2	0.84±0.11	1.00±0.38	2.60±0.24

Table 3 - Means and Standard Error of Means (SEM) of Days in Milk (DIM), number of lactations and somatic cell count (SCC) per Ultrasound Score (US Score).

Variables	US Score 0 (means±SEM)	US Score 1 (means±SEM)	US Score 2 (means±SEM)	US Score 3 (means±SEM)
DIM (days)	260.97±11.72	240.62±15.19	254.32±16.82	241.79±26.80
Number of lactations	3.41±0.28	3.30±0.39	3.96±0.59	4.36±0.39
SCC x 10^3 (cells/ml)	32.52±7.25	91.92±39.11	109.08±57.78	412.07±159.52

RESULTS

According to milk culture and clinical examination, 62 quarters have been classified as group CTR, 77 as group IMI, 7 as group SUB and 5 as group CL. Due to uncertain microbial culture, 2 quarters have been excluded. From the analysis, 73 quarters were positive for CoNS, 4 to *Bacillus* spp., 4 to *Streptococcus Uberis,* 3 to *Enterococcus* spp., 2 to *Escherichia Coli*, 1 to *Serratia* spp., 1 to *S. Aureus* and 1 to *Proteus* spp..

From the statistical analysis, means and SEM of DIM (expressed in days), number of lactations and SCC (expressed in cells/ml) of all the animals enrolled have been calculated. Results for DIM, number of lactations and SCC were respectively 253.11±7.81, 3.56±0.20, 94.94±21.70.

Of all ultrasound images performed of the mammary gland, 75 have been classified as score 0, 37 as score 1, 25 as score 2, 14 as score 3.

Different means and SEM of DIM, number of lactations, SCC and US Score have been expressed according to group CTR, IMI, SUB and CL. Results are reported in table 2.

Kruskal-Wallis rank sum test has then been applied in order to evaluate significative differences of DIM, number of lactations, SCC and US Score between groups. Results show significant differences both considering SCC and US Score (p-values < 0.01), with increasing values from group CTR to group CL.

Then, different means and SEM of DIM, number of lactations and SCC have been expressed according to different US Scores. Results are reported in table 3.

Kruskal-Wallis rank sum test has then been applied in order to evaluate significative differences of DIM, number of lactations and SCC between quarters having different ultrasound scores. Result shows significant difference of SCC between ultrasound score group, with p-value < 0.01.

DISCUSSION

Nowadays, mastitis still represents the most common cause of morbidity in adult dairy cows, causing economic losses due to therapy, reduction of milk production, milk discard, reproduction losses, and increased culling probability . Considering both the emerging problem of antimicrobial resistance and the massive usage of antimicrobials during mastitis, international pressure is applied to limit its application in animals, leading to the development of new preventive and therapeutic strategies, and new cow-side diagnostic tests. Ultrasonography represents a real-time technique already extensively used in cattle farms in several fields. More recent is its application to the evaluation of the mammary gland. Previous studies already assessed the ability of ultrasonography to estimate cisternal milk storage, and evaluate the percentage of secretory tissue and the numerical pixel value of parenchyma, both significantly correlated with daily milk yield. Moreover, correlation between non-homogeneous parenchyma during clinical mastitis and poor prognosis has been proved. The aim of our study was to evaluate ultrasonography as a reliable on farm tool able to identify mastitis in dairy cows. For the first trial, we focused on milk analysis and clinical evaluation related to the aspect of the mammary gland cistern, proposing an ultrasound score from 0 to 3 based on the echogenicity, as described in material and methods. Animals have been divided in 4 groups, based on clinical examination, microbial culture and SCC.

From microbial culture, 89 quarters out of 151 (58,9%) showed positivity. Moreover, 73 quarters were positive to CoNS, confirming high incidence of CoNS infections. Only 5 of the infected quarters showed clinical signs, with mammary swelling and milk alteration. These results agree with previous articles, assessing high prevalence of subclinical mastitis caused by CoNS in dairy cows.

No significant differences in DIM and parity were highlighted between groups. The non-significant effect of stage of lactation agrees with a study of 2019 analysing the impact of cow factors on intramammary infection. Differently, authors found significant difference between parity order. This divergence might be explained by a non-homogeneous distribution of number of lactations in our dataset.

From statistical analysis, a significant difference of SCC means between groups and between ultrasound scores, emerged. Particularly, SCC values increased from CTR to CL group and from score 0 to score 3. Moreover, also US score means increased gradually from CTR group to CL group, highlighting a significant difference. The difference of SCC between groups can be explained considering the physiopathology of the mastitis. SCC represents an important indicator of the health status of bovine

udders. Commonly, the cut-off value used to differentiate healthy and sick quarters is set to 200.000 cells/ml. Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection. SCC are mainly leukocytes (75 to 98%) and to a lesser extent epithelial cells (2-25%). Considering that mastitis represents an inflammatory and infective process, during its onset and development, SCC rapidly increase, due to the influx of neutrophils into the milk. Moreover, aggregation of leukocytes and blood clotting factors in milk can lead to the formation of clots, fibrin clumps and flakes. These structures can be well identified through ultrasound examination, as already assessed in several studies evaluating alterations during clinical mastitis, explaining also the significantly higher SCC levels in quarters having highest echogenicity.
The results of our study suggest the possibility to apply mammary gland cistern ultrasound for the detection of inflammatory processes and, consequently, high SCC. Obviously, this technique cannot replace laboratory examination such as microbiological culture and PCR. Nonetheless this technique can give a timely and fast indication on the mammary quarter health status, representing a possible important instrument in the management and detection of this widespread disease.
Further studies will focus on the validation of the US Score through texture examination, analyzing also parenchyma texture.

CONCLUSION

Mastitis, the inflammation of the mammary gland, represents one of the pathologies having the highest incidence in dairy cattle farms. Considering its impact both on the economy of the farm and on the welfare of the animals, a timely diagnosis is thus fundamental. The aim of this research was to evaluate ultrasound examination of the mammary gland cistern as a reliable on farm technique able to detect animals having subclinical mastitis. A preliminary ultrasound score has been set in order to establish an inflammatory grading based on the echogenicity of the cistern content. Quarters have been divided into CTR, IMI, SUB and CL based on SCC and microbial culture. Statistical analysis highlights a significant difference in SCC means both considering US Score and group. Particularly, increasing values of SCC have been detected rom CTR group to CL group and in quarters having US Score from 0 to 3. Moreover, also US Score means showed significant increasing values from CTR group to CL group. Considering the physiopathology of mastitis, these results suggest a possible application of ultrasound examination as a fast and immediate detection instrument of mastitis.
Further studies are needed in order to validate and deepen this technique.

Ethical approval

The study was conducted in accordance with the Euro-pean directive 2010/63/EU and the national law D.L.2014/26, and approved by Ethical Committee of the University of Padua (Prot. N. 20804/2024).

Author Contributions

Conceptualization, M.G. and C.T.; methodology, A.L.; writing-original draft preparation, C.T.; writing-review and editing, A.L., G.T., A.G., F.C. and E.F.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This study was carried out within the Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) - MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 - D.D. 1032 17/06/2022, CN00000022).

References

1. Klaas, I.C.; Zadoks, R.N. An Update on Environmental Mastitis: Challenging Perceptions. *Transbound Emerg Dis* **2018**, *65*, 166-185, doi:10.1111/TBED.12704.
2. Jamali, H.; Barkema, H.W.; Jacques, M.; Lavallée-Bourget, E.M.; Malouin, F.; Saini, V.; Stryhn, H.; Dufour, S. Invited Review: Incidence, Risk Factors, and Effects of Clinical Mastitis Recurrence in Dairy Cows. *J Dairy Sci* **2018**, *101*, 4729-4746, doi:10.3168/JDS.2017-13730.
3. Blosser, T.H. Economic Losses from and the National Research Program on Mastitis in the United States. *J Dairy Sci* **1979**, *62*, 119-127, doi:10.3168/jds.S0022-0302(79)83213-0.
4. Zecconi, A.; dell'Orco, F.; Rizzi, N.; Vairani, D.; Cipolla, M.; Pozzi, P.; Zanini, L. Cross-Sectional Study on the Prevalence of Contagious Pathogens in Bulk Tank Milk and Their Effects on Somatic Cell Counts and Milk Yield. *Ital J Anim Sci* **2020**, *19*, 66-74, doi:10.1080/1828051X.2019.1693282/ASSET/7F54DB76-C8C9-4D63-A3FB-AB5983685BFD/ASSETS/IMAGES/TJAS_A_1693282_F0001_B.JPG.
5. Barlow, J. Mastitis Therapy and Antimicrobial Susceptibility: A Multispecies Review with a Focus on Antibiotic Treatment of Mastitis in Dairy Cattle. *J Mammary Gland Biol Neoplasia* **2011**, *16*, 383-407, doi:10.1007/S10911-011-9235-Z/METRICS.
6. Zadoks, R.N.; Middleton, J.R.; McDougall, S.; Katholm, J.; Schukken, Y.H. Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans. *J Mammary Gland Biol Neoplasia* **2011**, *16*, 357-372, doi:10.1007/S10911-011-9236-Y/TABLES/2.
7. Tenhagen, B.A.; Köster, G.; Wallmann, J.; Heuwieser, W. Prevalence of Mastitis Pathogens and Their Resistance Against Antimicrobial Agents in Dairy Cows in Brandenburg, Germany. *J Dairy Sci* **2006**, *89*, 2542-2551, doi:10.3168/JDS.S0022-0302(06)72330-X.
8. Cobirka, M.; Tancin, V.; Slama, P. Epidemiology and Classification of Mastitis. *Animals 2020, Vol. 10, Page 2212* **2020**, *10*, 2212, doi:10.3390/ANI10122212.
9. Gruet, P.; Maincent, P.; Berthelot, X.; Kaltsatos, V. Bovine Mastitis and Intramammary Drug Delivery: Review and Perspectives. *Adv Drug Deliv Rev* **2001**, *50*, 245-259, doi:10.1016/S0169-409X(01)00160-0.
10. Bradley, A.J. Bovine Mastitis: An Evolving Disease. *Veterinary Journal* **2002**, *164*, 116-128, doi:10.1053/tvjl.2002.0724.
11. Sears, P.M.; McCarthy, K.K. Diagnosis of Mastitis for Therapy Decisions. *Veterinary Clinics of North America - Food Animal Practice* **2003**, *19*, 93-108, doi:10.1016/S0749-0720(02)00074-9.
12. Ashraf, A.; Imran, M. Diagnosis of Bovine Mastitis: From Laboratory to Farm. *Trop Anim Health Prod* **2018**, *50*, 1193-1202, doi:10.1007/S11250-018-1629-0/FIGURES/2.
13. Pyörälä, S. Indicators of Inflammation in the Diagnosis of Mastitis. *Vet Res* **2003**, *34*, 565-578, doi:10.1051/VETRES:2003026.
14. Bradley, A.; Green, M. Use and Interpretation of Somatic Cell Count Data in Dairy Cows. *In Pract* **2005**, *27*, 310-315, doi:10.1136/inpract.27.6.310.
15. Adkins, P.R.F.; Middleton, J.R. Methods for Diagnosing Mastitis. *Veterinary Clinics of North America - Food Animal Practice* **2018**, *34*, 479-491, doi:10.1016/j.cvfa.2018.07.003.
16. Tommasoni, C.; Fiore, E.; Lisuzzo, A.; Gianesella, M. Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives. *Animals 2023, Vol. 13, Page 2538* **2023**, *13*, 2538, doi:10.3390/ANI13152538.
17. Quintela, L.A.; Barrio, M.; Peña, A.I.; Becerra, J.J.; Cainzos, J.; Herradón, P.G.; Díaz, C. Use of Ultrasound in the Reproductive Management of Dairy Cattle. *Reproduction in Domestic Animals* **2012**, *47*, 34-44, doi:10.1111/J.1439-0531.2012.02032.X.

18. Giannuzzi, D.; Toscano, A.; Pegolo, S.; Gallo, L.; Tagliapietra, F.; Mele, M.; Minuti, A.; Trevisi, E.; Marsan, P.A.; Schiavon, S.; et al. Associations between Milk Fatty Acid Profile and Body Condition Score, Ultrasound Hepatic Measurements and Blood Metabolites in Holstein Cows. *Animals* **2022**, *12*, 1202, doi:10.3390/ANI12091202/S1.
19. Fiore, E.; Lisuzzo, A.; Beltrame, A.; Contiero, B.; Gianesella, M.; Schiavon, E.; Tessari, R.; Morgante, M.; Mazzotta, E. Lung Ultrasonography and Clinical Follow-Up Evaluations in Fattening Bulls Affected by Bovine Respiratory Disease (BRD) during the Restocking Period and after Tulathromycin and Ketoprofen Treatment. *Animals 2022, Vol. 12, Page 994* **2022**, *12*, 994, doi:10.3390/ANI12080994.
20. Franz, S.; Hofmann-Parisot, M.M.; Baumgarner, W. Evaluation of Three-Dimensional Ultrasonography of the Bovine Mammary Gland. *Am J Vet Res* **2004**, *65*, 1159-1163, doi:10.2460/AJVR.2004.65.1159.
21. Fasulkov, I.R. Ultrasonography of the Mammary Gland in Ruminants: A Review. *Bulg J Vet Med* **2012**, *15*, 1-12.
22. Themistokleous, K.S.; Sakellariou, N.; Kiossis, E. A Deep Learning Algorithm Predicts Milk Yield and Production Stage of Dairy Cows Utilizing Ultrasound Echotexture Analysis of the Mammary Gland. *Comput Electron Agric* **2022**, *198*, 106992, doi:10.1016/J.COMPAG.2022.106992.
23. Vang, A.L.; Dorea, J.R.R.; Hernandez, L.L. Graduate Student Literature Review: Mammary Gland Development in Dairy Cattle - Quantifying Growth and Development. *J Dairy Sci* **2024**, doi:10.3168/JDS.2024-25007.
24. Nishimura, M.; Yoshida, T.; El-Khodery, S.; Miyoshi, M.; Furuoka, H.; Yasuda, J.; Miyahara, K. Ultrasound Imaging of Mammary Glands in Dairy Heifers at Different Stages of Growth. *J. Vet. Med. Sci* **2011**, *73*, 19-24.
25. Flöck, M.; Winter, P. Diagnostic Ultrasonography in Cattle with Diseases of the Mammary Gland. *Veterinary Journal* **2006**, *171*, 314-321, doi:10.1016/J.TVJL.2004.11.002.
26. Suzuki, N.; Kurose, T.; Kaneko, S.; Haraguchi, A.; Isobe, N. Outcome Prediction from the First Examination in Clinical Mastitis Using Ultrasonography in Dairy Cows. *Animal Science Journal* **2020**, *91*, doi:10.1111/ASJ.13452.
27. Amorim, R.L.; Fonseca-Alves, C.E.; Palmieri, C.; Lucia, M.; Dagli, Z.; Mardones, F.O.; Barbagianni, M.S.; Gouletsou, P.G. Modern Imaging Techniques in the Study and Disease Diagnosis of the Mammary Glands of Animals. *Veterinary Sciences* **2023**, *10*, doi:10.3390/VETSCI10020083.
28. Mourya, A.; Shukla, P.C.; Gupta, D.K.; Sharma, R.K.; Nayak, A.; Tiwari, A.; Singh, B.; Singh, A.P.; Sahi, A.; Jain, A. Ultrasonographic Alteration in Subclinical Mastitis in Cows. *J Entomol Zool Stud* **2020**, *8*, 2058-2063.
29. Santos, V.J.C.; Simplício, K.; Sanchez, D.; Coutinho, L.; Teixeira, P.; Barros, F.; Almeida, V.; Rodrigues, L.; Bartlewski, P.; Oliveira, M.; et al. B-Mode and Doppler Sonography of the Mammary Glands in Dairy Goats for Mastitis Diagnosis. *Reproduction in Domestic Animals* **2015**, *50*, 251-255, doi:10.1111/RDA.12479.
30. IZSVe Diagnostica Di Laboratorio Nel Settore Bovino: Manuale Di Campionamento. **2012**.
31. Franz, S.; Hofmann-Parisot, M.; Baumgartner, W.; Windischbauer, G.; Suchy, A.; Bauder, B. Ultrasonography of the Teat Canal in Cows and Sheep. *Veterinary Record* **2001**, *149*, 109-112, doi:10.1136/VR.149.4.109.
32. Narayana, S.G.; Schenkel, F.; Miglior, F.; Chud, T.; Abdalla, E.A.; Naqvi, S.A.; Malchiodi, F.; Barkema, H.W. Genetic Analysis of Pathogen-Specific Intramammary Infections in Dairy Cows. *J Dairy Sci* **2021**, *104*, 1982-1992, doi:10.3168/JDS.2020-19062.
33. Sinha, R.; Sinha, B.; Kumari, R.; M.R, V.; Verma, A.; Gupta, I.D. Effect of Season, Stage of Lactation, Parity and Level of Milk Production on Incidence of Clinical Mastitis in Karan Fries and Sahiwal Cows. *Biol Rhythm Res* **2021**, *52*, 593-602, doi:10.1080/09291016.2019.1621064.
34. Dohoo, I.R.; Leslie, K.E. Evaluation of Changes in Somatic Cell Counts as Indicators of New Intramammary Infections. *Prev Vet Med* **1991**, *10*, 225-237, doi:10.1016/0167-5877(91)90006-N.
35. Sharma, N.; Singh, N.K.; Bhadwal, M.S. Relationship of Somatic Cell Count and Mastitis: An Overview. *Asian-Australas J Anim Sci* **2011**, *24*, 429-438.
36. Khan, M.Z.; Khan, A. Basic Facts of Mastitis in Dairy Animals: A Review. *Pakistan Veterinary Journal (Pakistan)* **2006**, *26*.