# Sonographic and histological udder parenchyma changes after intramammary infection of sheep with *Mycoplasma agalactiae*#

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## Summary

The sonographic findings of the udder parenchyma and udder lymph nodes in 30 lactating sheep after experimental infection with *Mycoplasma agalactiae* are described. The objective of the study was to describe infection related changes in the udder parenchyma and udder lymph nodes using physical, sonographic, and histological examination and to detect associations between sonographic and histological changes of the tissues. Animals were intramammarily infected with different mutant cocktails and the wild type PG2. One group served as a negative control. A 15 MHz linear transducer (Esaote MyLab 30 CV, Esaote, Florence, Italy) was used for sonographic examinations. Compared with the uninfected control group with homogeneously granular parenchyma, the udder lymph nodes were larger and the udder parenchyma was more inhomogeneous and partially hyperechoic. The corresponding histological findings in infected mammary glands comprised proliferation of interstitial connective tissue, non-purulent interstitial mastitis, and purulent galactophoritis. The infected udder lymph nodes showed reactive hyperplasia. The findings obtained in this study may improve the diagnosis of Mycoplasma mastitis in sheep.

**Keywords:** Sonography, histology, *Mycoplasma agalactiae*, udder parenchyma, udder lymph nodes

## Untersuchungen zu sonographischen und histologischen Euterparenchymveränderungen nach intramammärer Infektion von Schafen mit *Mycoplasma agalactiae*

Die sonographischen Befunde des Euterparenchyms und der Euterlymphknoten bei 30 laktierenden Schafen nach experimenteller Infektion mit *Mycoplasma agalactiae* werden beschrieben. Zielsetzung der Studie war die Beschreibung der infektionsbedingten Veränderungen in Euterparenchym und Lymphknoten mit Hilfe von klinischer, sonographischer und histologischer Untersuchung und der Vergleich von sonographischen und histologischen Veränderungen. Die Schafe wurden mit verschiedenen Mutanten-Cocktails und einer Positivkontrolle (Wildtyp PG2) intramammär infiziert. Eine Gruppe diente als Negativkontrolle. Für die Ultraschalluntersuchungen kam ein 15 MHz-Linear-Schallkopf (Esaote MyLab 30 CV, Esaote, Florenz) zum Einsatz. Im Vergleich zur nicht infizierten Kontrollgruppe mit homogen granulärem Parenchym waren die Euterlymphknoten grösser und das Euterparenchym inhomogener und teilweise echoreicher. Histologisch zeigten die infizierten Euterhälften neben einer Proliferation des interstitiellen Bindegewebes, eine nicht eitrige interstitielle Mastitis sowie eitrige Galaktophoritis. Die infizierten Lymphknoten wiesen eine reaktive Hyperplasie auf. Die in dieser Studie gewonnenen Erkenntnisse können die Mastitisdiagnostik bei Mykoplasmeninfektionen beim Schaf verbessern.

**Schlüsselwörter:** Ultraschall, *Mycoplasma agalactiae*, Histologie, Euterparenchym, Lymphknoten

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## Introduction

The «infectious agalactia» in small ruminants is caused by *Mycoplasma (M.) agalactiae* and some less common Mycoplasma species such as *M. mycoides subsp. capri, M. capricolum subsp. capricolum*, and *M. putrefaciens*. The disease occurs worldwide especially in countries with intensive sheep and goat farming. The economically important disease causes inflammation of udder tissue, joints, eyes, respiratory and reproductive tract. The massive decrease of milk yield is accompanied by changes in milk character, fibrosis, formation of abscesses, and enlargement of udder lymph nodes.[3]

Numerous pathogenic mycoplasmas possess multigene systems, which enable them to change their antigenic cell surface structure with unusually high frequency.[14] Such a gene locus with six related genes encoding the most important immundominant surface lipoproteins the Vpmas (variable proteins of *M. agalactiae*) was identified also in *M. agalactiae*. Xer1 recombinase is responsible for the site-specific recombinations occurring within the vpma gene locus. This surface variability presumably primarily results in immune evasion and host colonization and may be responsible for persistency and chronicity of the infection. By targeted gene disruption of the xer1 gene further Vpma switching was abolished and the phase-locked mutants continued to steadily express only a single Vpma product.[6] These were used in an experimental intramammary infection in lactating sheep to define Vpma oscillation under in vivo conditions in the natural host compared to wild-type PG2.[10,11] In this research project, sonography of the udder parenchyma was used to select healthy sheep. The present study was part of a larger project. Sonography was performed to supplement clinical and microbiological examination. The objective of the study was to describe infection related changes in the udder parenchyma and udder lymph nodes using physical, sonographic, and histological examination and to detect associations between sonographic and histological changes of the tissues.

The hypothesis of the study was that the changes in udder and lymphoid tissues can be visualized ultrasonographically, which may indicate a possible use of sonography in the diagnosis of Mycoplasma mastitis in sheep.

## Material and methods

Thirty Merino ewes from the University Teaching and Research Farm were selected, ranging in age from 1,8 to 2,3 years. The 30 sheep had been in first lactation for an average of 22,5 days (6 to 39 days) at the time of the initial physical examination. Animals were selected based on clinical,[2] sonographic,[4] and microbiological examination,[5] as well as somatic cell count determination (DCC cell counter, DeLaval, Tumba, Sweden) to ensure that the sheep did not have a mastitis and the udders were not infected by *M. agalactiae* or other pathogens. In the European Union the Regulation 853/2004 reported the criteria for hygienic production of milk and set the legal limit for cows at 400,000 cells/ml but no legal limit for this parameter is reported for sheep.

Animals were randomly assigned by alternate allocation into 5 groups of 6 animals each and intramammarily infected with different *M. agalactiae* mutant cocktails and the wild type PG2. One group served as a negative control. Groups were formed 4 days before infection and housed in the isolation pen at the University of Veterinary Medicine, Vienna. Infection was induced by instilling $10^9$ colony forming units (cfu) of *M. agalactiae* (mutant cocktails 1–3, wild tpe PG2) into the right teat. The negative control group received an identical volume of isotonic saline (0,9% NaCl).[10,11] During the study period of two weeks after infection, milk samples were taken from both udder halves at regular intervals (infection day 0: 2h, 4h, 8h, 12h and once at day 1, 2, 3, 5, 7, 9, 11, 13, 15) and examined microbiologically. The microbiological methods are described elsewhere.[5,9,10,11] Fifteen to 17 days after infection, the sheep were euthanized and tissue samples were collected from each udder half for bacteriologic and histologic examination. The lymphnodes were excised in toto. At this time, the second ultrasound examination of the udder parenchyma and udder lymph nodes was performed on the already sedated animals using a 15 MHz linear transducer (Esaote MyLab 30 CV, Esaote, Florence, Italy). At least 3 sonograms and numerous video clips (á 10 seconds) of each udder half were stored per animal. Measurements were made using cursors during the examination. The sonomorphology of the parenchyma was coded as follows: 0 = homogeneously granular, 1 = granular with thin hyperechoic septa, 2 = inhomogeneous, hyperechoic, 3 = honeycombed.

For the detection of mycoplasma the milk and tissue samples were cultured in Aluotto medium in ambient air at 37°C for 72 h and then sub-passaged on Aluotto agar at 37°C under 5% $CO_2$ atmosphere. By using a species-specific PCR the mycoplasmas detected by bacterial culture were identified as *M. agalactiae*. To determine the bacterial count of mycoplasmas in the examined samples, dilution series were prepared, plated on Aluotto agar media, and the colony-forming units per dilution step were counted.[5]

The collected udder samples were fixed in a 4% buffered formaldehyde solution (10% formalin) for at least 24 hours, then dehydrated and embedded in paraffin. A microtome was used to prepare sectional preparations with a thickness of approximately 4 – 5 µm, which were subsequently stained with the standard hematoxylin-eo-

sin stain and examined under a light microscope. For specific demonstration of mycoplasma, immunohistochemistry was performed using *M. agalactiae* specific rabbit polyclonal antiserum.[9]

## Results

**Clinical examination and determination of somatic cell count.**

All animals remained clinically healthy during the experimental period, with unaffected body temperature, pulse rate, respiratory rate, feed intake, rumen motility, and shedding of feces. The infected right udder halves showed, in addition to a massive decrease in milk yield at the latest from day 2 p. i., a high-grade increase in somatic cell counts and milk quality changes, with the milk first becoming thick and then serous, and showing yellow to gray color changes and flakes. Mean values of milk yield of right/left udder halves were 253,3 ± 115,6/260,8 ± 136,0 ml before infection, 12,3 ± 13,9/89,2 ± 69,7 ml each on day 2 after infection, and 1,6 ± 2,7/32,1 ± 52,8 ml on day 15.

The mean number of somatic cells per ml of milk was 68 ± 47 × $10^3$ on the right and 64 ± 33 × $10^3$ on the left before infection and 1636 ± 536 × $10^3$ on the right and 272 ± 587 × $10^3$ on the left on day 14 after infection. As early as day 2 after infection, cell counts averaged 1928 ± 590 × $10^3$ on the right and 49 ± 23 × $10^3$ on the left. The mean somatic cell count of animals in the negative control group were 139 ± 127 × $10^3$ /ml in the right and 91 ± 56 × $10^3$ /ml in the left before infection and 108 ± 51 × $10^3$ /ml in the right and 202 ± 122 × $10^3$ /ml in the left at day 14 after injection of 0,9 % NaCl solution.

Adspection and palpation findings on day 15 post infection showed differences between right (infected) and left (uninfected) udder halves, in terms of low-grade atrophy, firm-elastic consistency of all infected right halves and enlargement of all infected right udder lymph nodes.

**Bacteriological examinations**

Detection of *M. agalactiae* by bacterial culture in the milk samples was possible beginning 4 hours after infection until the end of the experiment after two weeks in each right half milk sample. *M. agalactiae* was also detected in udder tissue samples two weeks after infection what confirmed the experimental infection in all infection groups.

**Necropsy, histological and immunohistochemical examinations**

The right udder halves had a firm texture and multiple micro-abscesses were present in one animal of the positive control and 5 animals of the mutant group. Histologically, the right udders showed proliferation of interstitial connective tissue, moderate to severe non-purulent interstitial mastitis, and purulent galactophoritis (Figure 2). By immunohistochemical examination positive *M. agalactiae* antigen signals were detected in the udder tissues (Figure 3) and in the udder lymph nodes (Figure 4). The normal left udder parenchyma is shown in figure 1. In the mutant group in 10 animals a low-grade proliferation of interstitial connective tissue was present in the left half and among them 8 animals additionally had developed a moderate inflammatory round-cell infiltration. In the negative control group udder parenchyma and lymph nodes of all animals were macroscopically and histologically unremarkable on both sides. Histological and immunohistochemical findings in infected right and noninfected left udder halves are given in table 1.

**Sonographic examinations**

*Udder parenchyma*

The udder parenchyma appeared either homogeneously granular (Figure 5), granular and interspersed with thin hyperechoic septa (Figure 6), inhomogeneous hyperechoic (Figure 7) or «honeycombed» (Figure 8). However, a

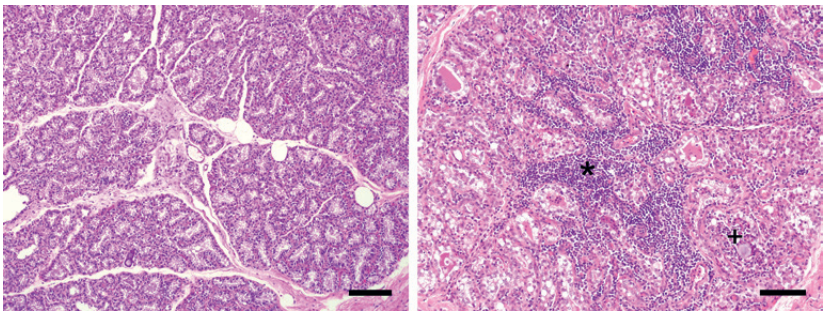



**Figure 1:** Photomicrograph of noninfected healthy udder parenchyma of sheep number 15. Hematoxylin and Eosin staining, bar = 150 µm.

**Figure 2:** Histologic appearance of infected udder parenchyma of sheep number 13 with non-purulent interstitial mastitis (*), and purulent galactophoritis (+) 17 days after intramammary infection with *Mycoplasma agalactiae*. Hematoxylin and Eosin staining, bar = 100 µm.

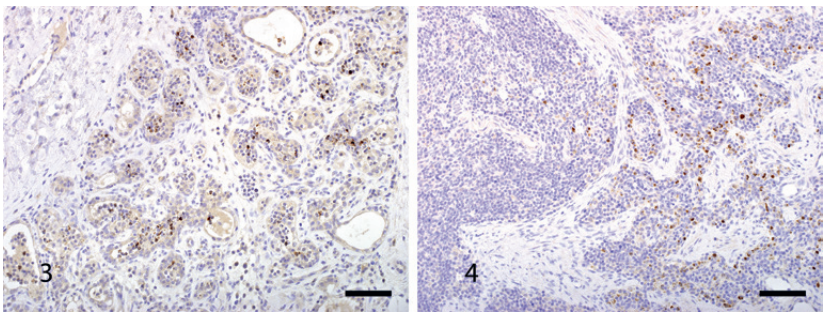



**Figure 3:** Immunohistochemical examination of udder tissue in sheep number 12 revealed positive *Mycoplasma agalactiae* antigen signals. Antigen *Mycoplasma agalactiae* immunohistochemical staining, bar = 80 µm.

**Figure 4:** Immunohistochemical examination of udder lymph nodes in sheep number 1 revealed positive *Mycoplasma agalactiae* antigen signals. Antigen *Mycoplasma agalactiae* immunohistochemical staining , bar = 80 µm.

**Table 1:** Histological and immunohistochemical findings in infected right (R) and noninfected left (L) udder halves 15-17 days after intramammary infection with *Mycoplasma agalactiae*.

| | Negative control R (n=6) | Negative control L (n=6) | Positive control R (n=6) | Positive control L (n=6) | Mutants R (n=18) | Mutants L (n=18) |
|---|---|---|---|---|---|---|
| proliferation of interstitial connective tissue | 0 | 0 | 6 | 2 | 18 | 10 |
| non-purulent interstitial mastitis | 0 | 0 | 6 | 0 | 18 | 0 |
| purulent galactophoritis | 0 | 0 | 6 | 0 | 18 | 0 |
| lymph node reactive hyperplasia | 0 | 0 | 6 | 0 | 18 | 5 |
| positive *M. agalactiae* antigen signals by immunohistochemical examination of udder tissue | 0 | 0 | 6 | 0 | 18 | 3 |
| positive *M. agalactiae* antigen signals by immunohistochemical examination of udder lymph nodes | 0 | 0 | 5 | 0 | 14 | 5 |

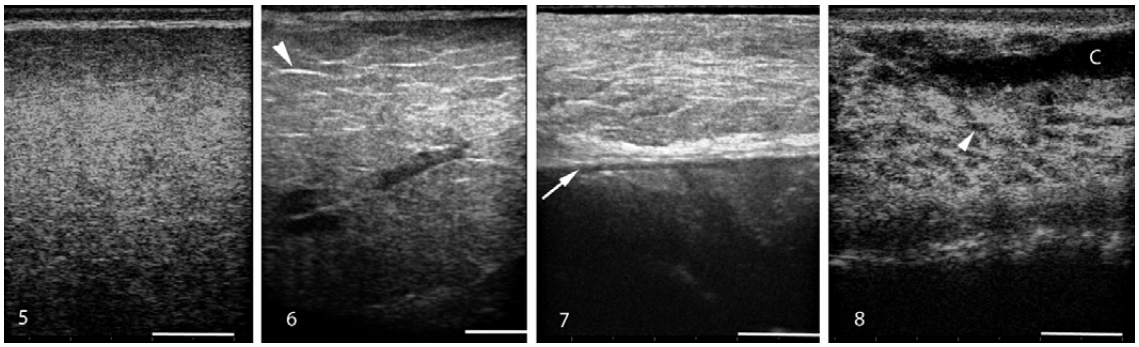
**Table 2:** Results of sonographic examination of mammary tissue in sheep 4 days before intramammary infection (1st examination) and 15–17 d after infection (2nd examination) with *Mycoplasma agalactiae*. An increase in score is indicated in bold numbers. Coding: homogeneously granular (0), granular and interspersed with thin hyperechoic septa (1), inhomogeneous hyperechoic (2), «honeycombed» (3)

| Group | Sheep No. | 1. Examination | | 2. Examination | |
|---|---|---|---|---|---|
| | | Right | Left | Right | Left |
| Mutants | 14 | 0 | 0 | **3** | **2** |
| | 2 | 1 | 1 | **3** | **3** |
| | 3 | 0 | 0 | **2** | **2** |
| | 4 | 0 | 0 | **3** | **2** |
| | 5 | 1 | 1 | **3** | **2** |
| | 6 | 3 | 1 | 3 | **2** |
| | 7 | 3 | 3 | 3 | 3 |
| | 8 | 1 | 1 | **3** | 1 |
| | 9 | 0 | 0 | **3** | **2** |
| | 4[a] | 1 | 1 | **2** | **2** |
| | 5[a] | 1 | 1 | **3** | 1 |
| | 6[a] | 1 | 1 | **2** | **2** |
| | 7[a] | 1 | 1 | **2** | 1 |
| | 8[a] | 1 | 1 | **3** | **3** |
| | 9[a] | 1 | 1 | **3** | 1 |
| | 10[a] | 1 | 1 | **2** | 1 |
| | 11[a] | 1 | 1 | **3** | 1 |
| | 12[a] | 3 | 1 | 1 | 1 |
| Positive control | 12 | 0 | 0 | **3** | **2** |
| | 13 | 1 | 1 | **3** | **3** |
| | 1 | 0 | 0 | **3** | **2** |
| | 13[a] | 1 | 1 | **3** | 1 |
| | 14[a] | 1 | 1 | **2** | 1 |
| | 15[a] | 0 | 1 | **2** | 0 |
| Negative control | 15 | 0 | 0 | **2** | **2** |
| | 10 | 2 | 2 | 2 | **3** |
| | 11 | 2 | 2 | 0 | 0 |
| | 1[a] | 1 | 1 | **2** | **2** |
| | 2[a] | 1 | 1 | **2** | **2** |
| | 3[a] | 1 | 1 | 1 | 1 |

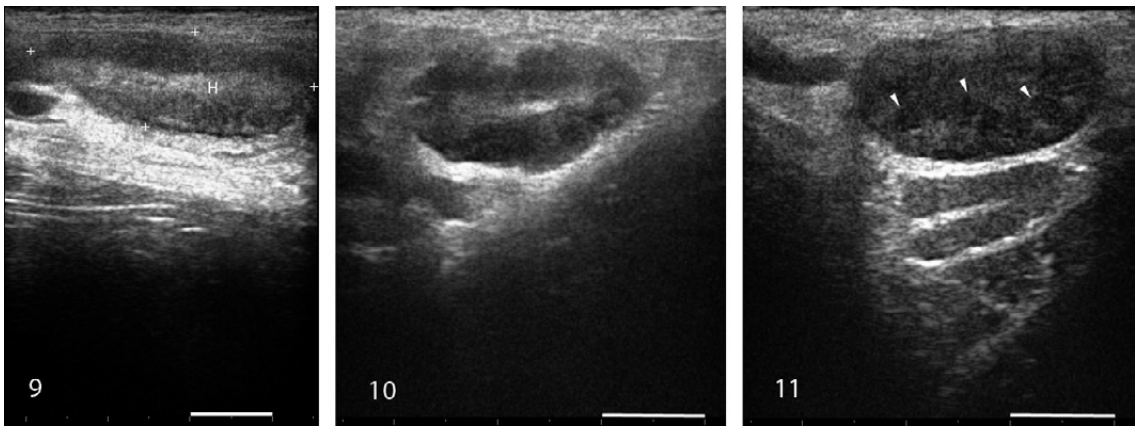
clear classification was not always possible because the changes of ultrasonographic appearance were fluent. In the mutant and positive control groups homogeneously granular parenchyma and granular parenchyma interspersed with thin hyperechoic septa was found in 21 right udder halves before and in 1 right udder half after infection. Inhomogeneous hyperechoic and «honeycombed» parenchyma was found in 3 right udder halves before and in 23 right udder halves after infection. The findings are shown in table 2.

*Udder lymph nodes*

During the first examination before infection 3 lymph nodes on the right and left showed an irregular surface (Figure 10); during the second examination after infection there were 7 on the right and 5 on the left with irregular surfaces. Hypechoic secondary follicles were visible in 5 lymph nodes on the right and 8 on the left during the first examination, and in 14 on the right and 8 on the left in the second examination (Figure 11). The sonogram of a physiological udder lymph node is shown in Figure 9. The dimensions of the udder lymph nodes are shown in table 3. The mean values of the length and width measurements obtained during the pathological-anatomical section were 3,6 and 10,9 mm, respectively, higher than those of the ultrasound measurements taken on the same day. An elongated oval to bean-shaped lymph node shape was found in 36 of 60 lymph nodes. The remaining ones had irregular shapes.




**Figures 5, 6, 7, and 8**: Ultrasonograms of healthy and inflamed udder parenchyma in sheep 17 days after intramammary infection with *Mycoplasma agalactiae.* A 15 MHz linear transducer was used for sonographic examinations. Left in figures is dorsal, right is ventral, bar = 1 cm. Figure 5 (sheep no. 4, 1st examination): Homogenous granular parenchyma of non-infected udder tissue. Figure 6 (sheep no. 5a, 1st examination): Granular udder parenchyma with thin hyperechoic septa (arrowhead) in non-infected udder tissue. The well distinguishable oval hypoechoic areas in the middle are blood vessels. Figure 7 (sheep no. 10a, 2nd examination): Inhomogenous hyperechoic udder parenchyma 17 days after infection, the horizontal hyerechoic structure is the septum between the udder halves (arrow). Figure 8 (sheep no. 5, 2nd examination): Honeycomb-structure of udder tissue 16 days after infection. The hypoechoic area is the udder cistern (C). The epithelium of the milk ducts (arrowhead) appears hypoechoic in comparison to the surrounding tissue what is most likely due to edema and infiltration granulocytes. The infected udder tissue is generally more echoic in comparison to healthy tissue.




**Figures 9, 10, and 11**: Ultrasonograms of udder lymph nodes before and 17 days after intramammary infection with *Mycoplasma agalactiae.* Figure 9 (sheep no. 5a, 1st examination): Normal left udder lymph node (between cursors) is homogeneous and sightly hypoechoic to the surrounding soft tissues. The hilar portion (H) of the node is hyperechoic. A vessel is seen deep to the node. Figure 10 (sheep no. 6a, 2nd examination): Udder lymph node with irregular surface 17 days after intramammary infection. Figure 11 (sheep no. 15a, 2nd examination): Udder lymph node with hypoechoic secondary follicles (arrowheads) 17 days after intramammary infection. The lymph node is more prominent because of reduced echogenicity.

## Discussion

*Udder parenchyma*

The glandular udder tissue is covered by the udder capsule. From there connective tissue septa penetrate into the interior of the glandular udder tissue. The milk producing tissue is divided into glandular lobules. Each lobule comprises a small mammary duct with associated alveoli and they are separated from each other by the interlobular connective tissue, fine strands of which surround the alveoli as intralobular connective tissue in the lobules.[17] The reduction of the glandular tissue during involution is accompanied by a marked increase in connective tissue. The epithelium is greatly flattened. The intra- and interlobular connective tissue makes up 83 % of the tissue after involution is complete.[17]

The lobular sonomorphologic structure consisting of the alveolar areas and the hyperechoic connective tissue is no longer representable with increasing involution. The parenchyma becomes more homogeneous and loses echogenicity. Structures with intermediate echogenicity include fat, ductal and lobular epithelia, intralobular and periductal connective tissue. Interlobular connective tissue, lamellae of the trunk fascia, and suspensory ligament of the mammary gland (*Ligamentum suspensorium uberis*) are more hyperechogenic structures.[16] The sonographic changes during involution reflect histological changes, such as there is a reduction in the size of the glandular epithelial cells. The decreased echogenicity could be due to the decreased number of glandular epithelial cells and proportional increase in adipose tissue.[16]

The findings of homogeneously granular (code = 0, Figure 5) and granular with thin hyperechoic septa (code = 1, Figure 6) were typically found during the examination before infection in both halves and during the second examination in the left uninfected udder halves. The homogeneously granular parenchyma corresponded to the physiological udder parenchyma described in cattle[7] and sheep.[1,12,16] Inhomogeneous hyperechoic (code = 2, Figure 7) and «honeycombed» parenchyma (code = 3, Figure 8) were found more frequently during the second examination in the infected right halves. Even in control halves some animals had an increase in score and even though both half groups had some worsening, this was more pronounced in infected halves. It can be assumed that the findings of inhomogeneous hyperechoic and «honeycombed» parenchyma correlated with proliferation of interstitial connective tissue and inflammatory cell infiltration, but could also be due in part to physiological tissue changes with increasing lactation duration and involution of the glandular tissue. Concurrent involution caused by the infection is likely, since lactation of the right infected halves almost ceased shortly after infection. The appearance of the «honeycombed» parenchyma showed certain similarities with the mastitis caused by *Trueperella pyogenes* in cattle.[7]

Overall, however, sonographic udder parenchyma changes between the two examination times or between the right infected and left non-infected udder half were rather less severe compared to the histological findings.

*Udder lymph nodes*

The udder lymph nodes (*Lnn. mammarii*) are located at the base of the udder in the form of two bean-shaped nodes of different sizes, in rare cases a third lymph node might be present.[17] Normal lymph nodes have a cortex of uniform width and appear sonographically as evenely surfaced, elongated, and anechoic. A hyperechoic internal zone (hilus) represents the sonographic correlate of central fat and connective tissue structures.[15] After infection the right lymph nodes in particular but also some left lymph nodes showed an irregular surface and hypoechoic secondary follicles more frequently in comparison to before. The *Lnn. mammarii* of the negative control group decreased in size during the observation period, which is likely to correspond to physiological involution. In the animals with *M. agalactiae* infection the size of lymph nodes on both sides increased between the first and the second examination.

**Table 3:** Sonographic measurement of udder lymph nodes (arithmetic mean [AM] and standard deviation [SD]) of infected sheep and negative control, 4 days before intramammary infection (1st examination) and 15–17 days after intramammary infection (2nd examination) with *Mycoplasma agalactiae.*

| Lymph node | Examination | Negative control AM ± SD | | Infected sheep AM ± SD | |
|---|---|---|---|---|---|
| Lenght right | 1st | 31,3 | ±10,0 | 24,8 | ±10,1 |
| | 2nd | 24,7 | ±8,0 | 33,2 | ±6,3 |
| Length left | 1st | 29,2 | ±7,9 | 29,3 | ±9,4 |
| | 2nd | 18,4 | ±5,8 | 31,5 | ±9,4 |
| Width right | 1st | 9,9 | ±3,0 | 12,0 | ±3,9 |
| | 2nd | 8,0 | ±1,1 | 12,2 | ±2,5 |
| Width left | 1st | 6,4 | ±0,4 | 11,5 | ±3,3 |
| | 2nd | 6,5 | ±0,3 | 10,4 | ±3,8 |

Reactive lymph node hyperplasia has also been described in cattle with *Mycobacterium smegmatis* infection[8] and in sheep with staphylococcal infections of the udder.[13] The differences between sonographic measurements and those with rulers on the freely dissected lymph nodes probably came from the irregular shape of some lymph nodes.

One limitation of this study is the short experimental duration of 15 to 17 days, since longer infection periods can probably be expected to result in even more pronounced tissue changes. Moreover in the period between selection and grouping of the sheep and start of the experiment mastitis due to other pathogens than Mycoplasma could have occurred. Ultrasonography does not replace bacteriological criteria or somatic cell counts but gives additional information on the status of the udder. Nevertheless, these findings can be helpful for the diagnosis of mastitis due to *Mycoplasma agalactiae* in sheep, as sonographic examination can also detect processes deep in the udder that are not detected by palpation.[12] In addition, sonography, in contrast to microbiological diagnostics and cell count determination, can show the extent and degree of tissue damage and thus improve the determination of prognosis. However further studies on this topic would be interesting with a larger number of animals.

## Ethical statement

Experiments were conducted according to the guidelines of the Ethics Committee of the University of Veterinary Medicine Vienna and the Austrian Federal Ministry for Science and Research (approval numbers BMWF-68.205/002-II/3b/2011 and BMWF-68.205/0104-II/3b/2012).

## Modifications échographiques et histologiques du parenchyme de la mamelle après infection intramammaire de moutons par Mycoplasma agalactiae

Les constatations échographiques sur le parenchyme de la mamelle et des ganglions lymphatiques de la mamelle chez 30 brebis en lactation après une infection expérimentale avec Mycoplasma agalactiae sont décrits. L'objectif de l'étude était de décrire les modifications liées à l'infection dans le parenchyme mammaire et les ganglions lymphatiques de la mamelle à l'aide d'un examen physique, échographique et histologique et de détecter les associations entre les altérations échographiques et histologiques des tissus. Les animaux ont été infectés par voie intramammaire avec différents cocktails de mutants et le type sauvage PG2. Un groupe a servi de contrôle négatif. Une sonde linéaire de 15 MHz (Esaote MyLab 30 CV, Esaote, Florence, Italie) a été utilisé pour les examens échographiques. Comparativement au groupe témoin non infecté avec un parenchyme granulaire homogène, les ganglions lymphatiques de la mamelle étaient plus gros et le parenchyme de la mamelle était plus inhomogène et partiellement hyperéchogène. Les résultats histologiques correspondants dans les glandes mammaires infectées comprenaient une prolifération de tissu conjonctif interstitiel, une mammite interstitielle non purulente et une galactophorite purulente. Les ganglions lymphatiques de la mamelle infectée présentaient une hyperplasie réactive. Les résultats obtenus dans cette étude peuvent améliorer le diagnostic de la mammite à Mycoplasma chez le mouton.

**Mots clés:** Échographie, histologie, Mycoplasma agalactiae, parenchyme mammaire, ganglions mammaires

## Cambiamenti ecografici e istologici del parenchima della mammella dopo l'infezione intramammaria delle pecore con Mycoplasma agalactiae

In questo studio sono descritti i risultati ecografici del parenchima e dei linfonodi della mammella in 30 pecore in lattazione dopo l'infezione sperimentale con *Mycoplasma agalactiae*. L'obiettivo dello studio era di descrivere i cambiamenti legati all'infezione nel parenchima della mammella e nei linfonodi attraverso l'esame clinico, ecografico e istologico e di rilevare le associazioni tra i cambiamenti sonografici e istologici dei tessuti. Gli animali sono state infettati per via intramammaria con diversi cocktail mutanti e un controllo positivo (PG2 di tipo selvatico). Un gruppo è servito da controllo negativo. Un trasduttore lineare da 15 MHz (Esaote MyLab 30 CV, Esaote, Firenze, Italia) è stato utilizzato per gli esami ecografici. Rispetto al gruppo di controllo non infetto con parenchima granulare omogeneo, i linfonodi della mammella risultavano più grandi e il parenchima della mammella era più disomogeneo e parzialmente più iperecoico. I risultati istologici corrispondenti alle ghiandole mammarie infette hanno mostrato proliferazione del tessuto connettivo interstiziale, mastite interstiziale non purulenta e galattoforite purulenta. I linfonodi infetti hanno mostrato un'iperplasia reattiva. Le conoscenze acquisite in questo studio possono migliorare la diagnosi della mastite nelle infezioni da Micoplasma nelle pecore.

**Parole chiave:** ecografia, istologia, Mycoplasma agalactiae, parenchima della mammella, linfonodi

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