EC and CMT detect subclinical mastitis in dairy sheep but less sensitive than in dairy cows

Kerstin Barth[1], Elke Burow[2] and Karin Knappstein[3]

Abstract

Reliable tests to monitor the udder health of ewes are necessary to ensure a high raw milk quality in organic dairy sheep farms. 328 udder halves of 164 ewes from six organic farms were sampled in 2005. Foremilk samples were used to measure the electrical conductivity (EC) of the milk and for California Mastitis Test (CMT). Afterwards samples for cyto-bacteriological examination were aseptically collected. Pathogens were detected in 31 samples. In 24 cases coagulase-negative staphylococci caused the infection. Half udder samples free from pathogens contained 61.000 cells ml^{-1} on average. According to the recommendations of the German Veterinary Association (DVG, 2002) for the evaluation of cytobacteriological results of cow milk samples, 73.0 % of the halves were healthy and 5.3 % had a subclinical mastitis. CMT values corresponded with the measured somatic cell count ($r = 0.53$, $p < 0.01$) and the mean EC difference between healthy and udder halves with subclinical mastitis was 0.4 mS cm^{-1}, nearly similar to the well known threshold for mastitis detection in cows.

Besides the bacteriological analysis we recommend to use 100,000 cells ml^{-1} as a threshold to discriminate between healthy and mastitic halves and we have to say the common cow side tests are not so sensitive when applied to sheep milk.

Keywords: mastitis, dairy sheep, somatic cell count, electrical conductivity, organic farming

Zusammenfassung

Auch bei Schafen kann man mit der elektrischen Leitfähigkeit und dem Schalmtest subklinische Mastitis erkennen - jedoch weniger genau als bei Kühen

Die Sicherung einer hohen Milchqualität auch auf ökologisch wirtschaftenden Milchschafbetrieben erfordert verlässliche Verfahren zur Eutergesundheitsüberwachung. Im Jahr 2005 wurden 328 Euterhälften von 164 Milchschafen, die auf sechs Öko-Betrieben gehalten wurden beprobt. An den Vorgemelksproben wurde die elektrische Leitfähigkeit (LF) der Milch gemessen und der Schalm-Mastitis-Test (SMT) ausgeführt. Für die zytobakteriologische Untersuchung wurden anschließend Anfangsgemelkproben steril gewonnen. In 31 Proben konnten Erreger nachgewiesen werden. In 24 Fällen wurde die Infektion von koagulasenegativen Staphylokokken verursacht. Der mittlere Zellgehalt von bakteriologisch negativ getesteten Proben betrug 61.000 Zellen ml^{-1}. Wurden die Euterhälften entsprechend den Empfehlungen der Deutschen Veterinärmedizinischen Gesellschaft (DVG, 2002) für Kuhmilch klassifiziert, so waren 73,0 % aller Hälften als gesund und 5,3 % als subklinisch an Mastitis erkrankt einzustufen. Die Ergebnisse des SMT wiesen eine Beziehung zur somatischen Zellzahl auf ($r = 0{,}53$, $p < 0{,}01$). Die LF-Differenz zwischen gesunden und subklinisch erkrankten Hälften betrug durchschnittlich 0,4 mS cm^{-1} und entsprach damit annähernd dem in der Milchkuhhaltung angewendeten Grenzwert.

Für die Differenzierung zwischen gesunden und an Mastitis erkrankten Euterhälften bei Milchschafen empfehlen wir, neben der Durchführung der bakteriologischen Untersuchung, einen Zellzahlgrenzwert von 100,000 Zellen ml^{-1} anzuwenden. Die im Milchkuhbereich üblichen Schnelltests sind bei Milchschafen nur eingeschränkt aussagefähig.

Schlüsselwörter: Mastitis, Milchschafe, Zellzahl, Elektrische Leitfähigkeit, Ökologischer Landbau

1 Johann Heinrich von Thünen-Institut, Federal Research Institute for Rural Areas, Forestry and Fisheries (vTI), Institute of Organic Farming, Trenthorst 32, 23847 Westerau/Germany; Email: kerstin.barth@vti.bund.de

2 University Kassel-Witzenhausen, Steinstr. 19, 37213 Witzenhausen/Germany

3 Max Rubner-Institute, Federal Research Institute of Nutrition and Food (MRI), Department of Safety and Quality of Milk and Fish Products, Hermann-Weigmann-Str. 1, 24103 Kiel/ Germany

1 Introduction

Infection of the udder is one of the most important diseases in dairy production. This concerns not only dairy cows but also dairy ewes. Tremendous losses of milk yield or a deteriorated raw milk quality might be caused by mastitis of lactating ewes (Behrens et al., 2001; Jaeggi et al., 2003; Bianchi et al., 2004). Cases of subclinical mastitis are of particular importance due to the fact that they can not be identified by the visual evaluation of the foremilk.

In cows the definition of udder health is based on cyto-bacteriological analysis of quarter milk samples. Since 1994 the threshold of 100,000 cells ml^{-1} is used for somatic cell count (Table 1). Although it is required to apply this scheme only if three consecutive samples were gained, under a practical point of view it might be used for single samples, too, as long as the error rate will be considered.

Table 1:
Mastitis classification system recommended by DVG (1994)

	Somatic cell count [1,000 ml^{-1}]	
Pathogens*	≤ 100	> 100
not detected	normal secretion	unspecific mastitis
detected	latent infection	mastitis

* base: the same pathogen was detected in two out of three samples

However, a clear definition of udder health based on these parameters is still lacking in ewes. It is assumed that a higher somatic cell count (SCC) is physiological in sheep (Travniček & Federič, 1994; Tröger et al., 2003; Behrens et al., 2001). In contrast to the milk of goats, which contains cytoplasmatic particles making the automatic measurement difficult, sheep's milk contains the same classes of somatic cells as the milk of cows. Nevertheless, as long as no acute clinical mastitis occurs most of the farmers are afraid of the costs of a continuous monitoring by using cyto-bacteriological analyses. As an easy method SCC might be estimated using the well known California Mastitis Test (CMT). According to Winter (1998) and to McDougall et al. (2001) there is a positive and significant relationship between CMT and SCC in sheep milk, but these studies included cases of clinical mastitis. Thus, the question of the reliability for subclinical mastitis still remained. Honegger (1994) identified only 49 % of the infections using the CMT. However, that study involved only 11 ewes.

A second method to indicate subclinical mastitis without a laboratory analysis is the measurement of electrical conductivity (EC) of milk. Damages of the udder tissue cause an exchange of ions in the milk with an increase of sodium and chloride content leading to a higher EC (Haasmann & Schulz, 1994). Thus, EC of milk is not necessarily connected with the SCC: physical strain of the tissue might cause an increase of EC as well as pathogens (Barth, 2005). EC is negatively correlated with the milk fat content (Prentice, 1992). High fat contents constrain the movement of ions and the EC decreases. Peaker (1978) recommended comparing the EC readings of the udder quarters of the animal to achieve a high sensitivity. Due to the differences in milk composition and udder anatomy it is not clear if the findings made in cow's milk might be transferred to sheep.

This study aimed at an evaluation of CMT and EC measurement to monitor subclinical mastitis in sheep and was based on an investigation of the udder health status in German organic farms.

2 Materials and methods

Milk samples were gained once in six organic dairy sheep flocks during morning or evening milking in May, June or September 2005. Four of the herds were located in the middle and two in the North of Germany. 25 to 150 ewes were kept in the herds and a maximum of 30 animals per herd was randomly selected for sampling (Table 2). Animals with clinical mastitis were excluded.

Table 2:
Characteristics of the investigated flocks

Farm	Animals/herd	Sampled	Breed
1	30	25	Ostfriesian dairy sheep (black)
2	26	19	Ostfriesian dairy sheep (black)
3	150	30	Ostfriesian dairy sheep (white)
4	48	30	Ostfriesian dairy sheep (white)
5	44	30	Lacaune
6	60	30	Crossbreed (Ostfriesian dairy sheep x Lacaune)

Sampling always started with the left udder half. First squirts were used for EC measurement to avoid the well known effect of alveolarmilk ejection on the readings. EC was measured with a handheld conductometer (Mastitron® plus V, MILKU), which needs only 6 ml milk and compensates the measured EC on a standard temperature of 25 °C. After measuring EC the milk was transferred to the test plate for CMT. CMT was always carried out by the same person to improve the reliability of the evaluation. CMT scores were 0, +, ++ and +++ for "negative", "weak positive", "positive" or "strong positive", respectively. After cleaning the teats with towels soaked in 98 % alcohol 10 ml-samples for cytobacteriological analyses were gained aseptically. Afterwards an additional sample of 30 ml for analyses of milk composition was taken. After all the animals were machine milked according to the farm's routine.

Samples were stored and transported at 6 to 8 °C. Cytobacteriological analyses were carried out by the laboratory of the MRI in Kiel. SCC was measured by Fossomatic 360 and Fossomatic 5000 (A/S N Foss Electric, Hillerød, DK) according to IDF standard 148A: 1995, method C (IDF, 1995). Isolation, identification and quantification of mastitis pathogens followed the standard of German Veterinary Association (DVG, 2000). A sample was defined as tested positive for pathogens if at least 21 colony forming units (CFU) of the same pathogen were detected in 0.05 ml. In negative samples a pathogen could not be isolated. If more than two different pathogens or less than 21 CFU were detected the sample was considered as contaminated.

Fat and lactose contents were measured by infrared spectroscopy (Milkoscan FT 6000, A/S N Foss Electric, Hillerød, Denmark) at the laboratory of Landeskontrollverband Schleswig-Holstein, also located in Kiel.

SPSS 11.5 and 12.0 were used for statistical examinations. Calculations concerning SCC were based on Log10 transformation of SSC. Spearman's rank correlation coefficient was calculated for the relation between SCC and CMT. Measures of dispersion were expressed as mean and standard deviation.

3 Results and discussion

328 udder half samples were gained from 164 ewes. Animals were 10 to 270 days in milk (DIM) and in their first to ninth lactation. 10 samples for cytobacteriological analyses were contaminated and thus excluded from the statistical analysis. In 90.3 % of the 318 remaining samples no pathogen could be isolated. The prevalence of infection was very low compared to the results of Gonzalo et al. (2002), who detected an infection in 24.6 % of all sampled udder halves. The prevailing pathogens were coagulase-negative staphylococci: 7.5 % of all samples contained CNS. This goes along with the results of other studies (Honegger, 1994; Wittek et al., 1998; Ariznabarreta et al., 2002; Winter, 2003), which found CNS as the leading pathogen of udder infection in ewes. Only in 3 samples (0.9 %) major pathogens were detected: one sample each contained *Staphylococcus aureus, Streptococcus dysgalactiae* or Esculin-positive streptococci.

The SCC of 77.1 % of the 328 samples was lower or equal to 100,000 cells per ml milk. The geometric mean over all samples was 73,000 cells per ml. The prevalence of specific mastitis in the investigated herds was low. Only 5.3 % of all halves had an increased SCC caused by an infection (Table 3). The total amount of subclinical cases (unspecific and specific mastitis) was 22.6 % and corresponded with the findings by Gonzalo et al. (2002) who found 24.6 %, and was within the range from 10 to 25 % which was defined as normal by Behrens et al. (2001). Only a few udder halves showed SCC exceeding 1,000,000/ml (Figure 1). Last square means of SCC of uninfected and infected halves were 61,000 and 341,000 cell ml^{-1}, respectively. The difference was significant ($p < 0.05$).

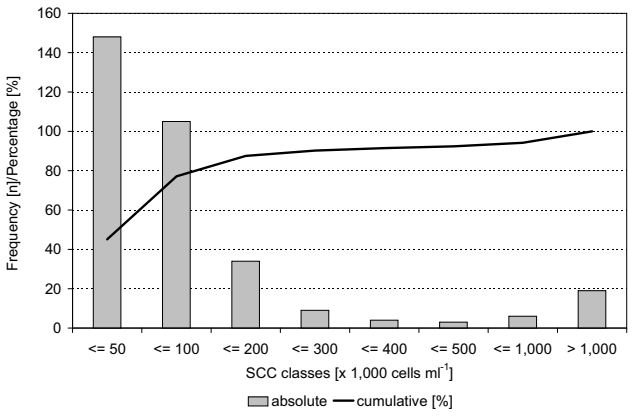

Figure 1:

Absolute frequency and cumulative percentage of samples (n=328) in classes of somatic cell count (SCC)

In view of these results, the usually recommended threshold of 500,000 cells ml^{-1} (Travniček & Federič, 1994; Behrens et al., 2001; Tröger et al., 2003) seems to be too high to differentiate between healthy halves and halves with subclinical mastitis.

In respect to the very few infected halves and the low SCC, the status of udder health in the herds was very

Table 3:

Distribution of udder halves of ewes according to categories of udder health status based on quarter milk samples of dairy cows by DVG (1994)

			Frequency	
Status	**SCC [1,000 ml $^{-1}$]**	**Pathogens**	**n**	**%**
normal secretion	≤ 100	not detected	232	73.0
latent infection	≤ 100	detected	14	4.4
unspecific mastitis	> 100	not detected	55	17.3
specific mastitis	> 100	detected	17	5.3
		Total	318	100.0

Table 4:
Comparison between classes of SCC and CMT score

	Somatic cell count [1,000 ml^{-1}]				
CMT Score	**≤ 100**	**≤ 500**	**≤ 1,000**	**> 1,000**	**Total**
0	244 (74.4)	33 (10.1)	0 (0.0)	0 (0.0)	277 (84.5)
+	6 (1.8)	10 (3.1)	1 (0.3)	0 (0.0)	17 (5.2)
++	1 (0.3)	3 (0.9)	4 (1.2)	1 (0.3)	9 (2.7)
+++	2 (0.6)	4 (1.2)	1 (0.3)	18 (5.5)	25 (7.6)
Total	253 (77.1)	50 (15.2)	6 (1.8)	19 (5.9)	328 (100.0)

Table 5:
Characteristics of samples from udder halves of dairy ewes

		n	**Mean**	**SD**	**Min**	**Max**
SCC	[1,000 ml^{-1}]	328	73	3.47	10	10,000
EC	[mS cm^{-1}]	328	5.0	0.53	3.7	8.6
Fat	[%]	319*	6.09	2.05	2.18	15.99
Protein	[%]	319*	5.00	0.48	4.09	6.67
Lactose	[%]	319*	5.13	0.54	4.33	7.82

* milk yield of 9 udder halves was to low

good. The relatively high amount of halves with unspecific mastitis might be explained with the decrease of SCC after a successful defence against an infection or the beginning of a reaction during an infection. Furthermore it might be possible that rare pathogens could not be detected by the applied standard method. In the study of Gonzalo et al. (2002) all cases without an infection had a SCC below 200,000 cells ml^{-1}.

As expected, CMT was highly significant correlated with SCC (r = 0.53) and confirmed the results of Wittek et al. (1998), Winter (1998) and McDougall et al. (2001).

96.4 % of the cases with a SCC ≤ 100,000 cells ml^{-1} were classified by CMT as "negative" and in 94.7 % of all samples containing over 1,000,000 cells ml^{-1} CMT showed a clear reaction (Table 4). Consistent with Schalm (1960), the range between 100,000 and 500,000 cells ml^{-1} was not clearly to define.

However, the CMT is a useful tool to monitor the udder health not only of dairy cows but also of dairy sheep. Main advantages of the CMT are the easy handling and the low costs (Wittek et al., 1998). Good results will be achievable if always the same person carries out the tests, because the evaluation might be influenced by changing persons especially in the most interesting range (Hamann, 1986).

Mean EC of all samples (n = 328) was 5.0 ± 0.56 mS cm^{-1} with a wide variation between 3.7 and 8.6 mS cm^{-1} (Table 5) due to different fat content. Calculation of EC differences between the halves of the udders resulted in a mean difference of 0.1 or 0.4 mS cm^{-1} for udders with two healthy (n = 96) or at least one half with specific mastitis (n = 6), respectively. This is in agreement with Fahr et al. (2003) and only 0.1 mS cm^{-1} lower than the threshold recommended to detect quarters with subclinical mastitis in cows (Schulz, 1994). Maybe the reduced number of comparable readings (udder halves instead of udder quarters) impacted the sensitivity.

4 Conclusions

The status of udder health in the investigated herds was better than expected by considering the literature research.

The mastitis classification system (DVG, 2002) which is used for cows was useful to evaluate milk samples of dairy sheep, too. As in cows, we suggest to use the threshold of 100,000 cells ml^{-1} to indicate subclinical mastitis. But the threshold should be tested using a larger number of animals.

To make a preselection und thus to reduce the costs for the cyto-bacteriological analysis of milk samples, it seems helpful to apply the CMT or to measure the EC of foremilk. A consistent documentation of the results per animal will give a good overview of the udder health status in the herd and will support other management decisions.

Acknowledgement

We would like to thank all participating farmers for helpfulness and patience during the days of milk sampling.

References

Ariznabarreta C, Gonzalo C, San Primitivo F (2002) Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. J Dairy Sci 85:1370-1375

Barth K (2005) Differences in milk conductivity on quarter level induced by milking machine. ICAR Technical series 10:123-127

Behrens H, Ganter M, Hiepe T (2001) Mastitis. In: Behrens H, Ganter M, Hiepe T Lehrbuch der Schafkrankheiten. Berlin : Parey, pp 87-92

Bianchi L, Bolla A, Budelli E, Caroli A, Casoli C, Pauselli M, Duranti E (2004) Effect of udder health status and lactation phase on the characteristics of Sardinian ewe milk. J Dairy Sci 87:2401-2408

Deutsche Veterinärmedizinische Gesellschaft (1994) Leitlinien zur Bekämpfung der Mastitis des Rindes als Bestandsproblem. Gießen : DVG, 78 p

Deutsche Veterinärmedizinische Gesellschaft (2000) Leitlinien zur Entnahme von Milchproben unter antiseptischen Bedingungen und Leitlinien zur Isolierung und Identifizierung von Mastitiserregern. Gießen : DVG, 57 p

Deutsche Veterinärmedizinische Gesellschaft (2002) Leitlinien zur Bekämpfung der Mastitis des Rindes als Bestandsproblem. Gießen : DVG, 126 p

Fahr RD, Schulz J, Kaskous S (2003) Merkmale der Eutergesundheit und deren Wechselbeziehungen bei Ostfriesischen Milchschafen : 8. DVG-Tagung der Fachgruppe „Krankheiten der kleinen Wiederkäuer" am 24./25.06.2003 in Grub bei München ; Vortragszusammenfassung. Tierärztl Prax 31(G):61

Gonzalo C, Ariznabarreta C, Carriedo JA, San Primitivo F (2002) Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. J Dairy Sci 85:1460-1467

Hamann J (1986) Vergleichende Untersuchungen von Zellgehalt und Leitfähigkeit in Viertelanfangsgemelksproben. Milchwissenschaft 41:8-11

Haasmann S, Schulz J (1994) Erkrankungen der Milchdrüse des Rindes : labordiagnostische Untersuchungen. In: Wendt K, Bostedt H, Mielke H, Fuchs H-W (eds) Euter- und Gesäugekrankheiten. Jena : Fischer, pp 269-279

Honegger R (1994) Eutergesundheit beim Milchschaf. Zürich : Univ, 71 p [Dissertation)

IDF (1995) Milk : enumeration of somatic cells. Brussels : International Dairy Federation, pp1-6

Jaeggi JJ, Govindasamy-Lucey S, Berger YM, Johnson ME, MCKusick BC, Thomas DL, Wendorff WL (2003) Hard ewe's milk cheese manufactured from milk of three different groups of somatic cell counts. J Dairy Sci 86:3082-3089

MCDougall S, Murdough P, Pankey W, Delaney C, Barlow J, Scruton D (2001) Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. Small Ruminant Res 40:245-254

Peaker M (1978) The electrical conductivity of milk for the detection of subclinical mastitis in cows : comparison of various methods of handling conductivity data with the use of cell counts and bacteriological examination. Br Vet J 134:308-314

Prentice JH (1962) The conductivity of milk – the effect of the volume and degree of dispersion of the fat. J Dairy Res 29:131-139

Schalm OW (1960) Ein neuer Mastitis-Test. Tierärztl Umsch 15:151-153

Schulz J (1994) Erkrankungen der Milchdrüse des Rindes : Diagnose und Prognose. In: Wendt, K, Bostedt H, Mielke H, Fuchs H-W (eds.) Euter- und Gesäugekrankheiten. Jena : Fischer, pp 280-284

Travnicek M, Federic F (1994) Euterkrankheiten der kleinen Wiederkäuer. In: Wendt, K, Bostedt H, Mielke H, Fuchs H-W (eds) Euter- und Gesäugekrankheiten. Jena : Fischer, pp 435-443

Tröger F, Schulz J, Lengerken G von, Fahr RD, Rodehutscord M, Rost D, Heinrich J (2003) Schaf und Ziege : Besonderheiten von Bau des Euters und Physiologie der Laktation. In: Fahr RD, Lengerken G von (eds) Milcherzeugung : Grundlagen, Prozesse, Qualitätssicherung. Frankfurt a M : Deutscher Fachverl, pp 593-595

Winter P (1998) Aspekte zur Diagnostik von Schafmilchproben. In: 6. DVG-Tagung der Fachgruppe „Krankheiten der kleinen Wiederkäuer" zum Thema „Schaf- und Ziegenkrankheiten" am 08.05.1998 in Gießen. Gießen : DVG, pp 34-40

Winter P (2003) Eutergesundheit bei Milchschafen und –ziegen. Tierärztl Prax 31(G):61

Wittek T, Elze K, Beck K (1998) Milchinhaltstoffe, Zellgehalt, klinische und bakteriologische Euterbefunde im Verlauf der Laktation in einer Milchschafherde. Prakt Tierarzt 79:762-768