VETERINARSKI ARHIV 86 (4), 475-485, 2016

Assessment of the Zagreb mastitis test in diagnosis of subclinical mastitis in dairy cattle

Nino Maćešić[1]*, Goran Bačić[1], Katarina Božičević[2], Miroslav Benić[3], Tugomir Karadjole[1], Nikica Prvanović Babić[1], Martina Lojkić[1], Maša Efendić[4], Iva Bačić[5], and Marina Pavlak[6]

[1]*Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Commodatio, Zagreb, Croatia*

[3]*Croatian Veterinary Institute, Zagreb, Croatia*

[4]*Veterinary School, Zagreb, Croatia*

[5]*Student, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

[6]*Department of Veterinary Economics and Analytic Epidemiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

MAĆEŠIĆ, N., G. BAČIĆ, K. BOŽIČEVIĆ, M. BENIĆ, T. KARADJOLE, N. PRVANOVIĆ BABIĆ, M. LOJKIĆ, M. EFENDIĆ, I. BAČIĆ, M. PAVLAK: Assessment of the Zagreb mastitis test in diagnosis of subclinical mastitis in dairy cattle. Vet. arhiv 86, 475-485, 2016.

ABSTRACT

The objective of this research was to evaluate the Zagreb mastitis test (ZMT) as screening test for mastitis diagnosis in dairy cows. The study was carried out within the framework of subclinical mastitis monitoring in the Varaždin region. Research included 1549 quarter milk samples from 389 Simmental dairy cows. Milk samples for bacteriological examination, mastitis testing and SCC were taken in a sterile plastic tube during milking. Bacteriology examination of milk samples obtained 760 (49.06%) positive samples. The most common isolated pathogens, *Staphylococcus aureus, Streptococcus* spp. Lancefield group D, *Pseudomonas aeruginosa,* were found in 388 (51.05%), 84 (11.05%), 62 (8.16%) positive quarter samples, respectively. Using a cut-off of ZMT by scores ≥ 1 as positive, 63.20% (CI 95%, 60.80% - 65.60%) quarter milk samples were estimated as being positive for subclinical mastitis. This cut-off had quite high sensitivity (96.84%, CI 95%, 95.60% - 98.06%) and negative predictive value (95.79%, CI 95%, 94.14% - 97.44%), however specificity (69.20%, CI 95%, 65.98% - 72.74%) and positive predictive value (75.17%, CI 95%, 72.47% - 77.86%) were low. Using a ZMT cut-off $\geq 4\times10^5$ cell/mL as positive, 49.56% of the quarters were positive. Sensitivity (88.37%, CI 95%, 86.06% - 90.68%) and specificity (86.98%, CI 95%, 84.57% - 89.38%), as well as predictive values, were

*Corresponding author:

Nino Maćešić, PhD, DVM, Clinic for Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 169 Fax: +385 1 2441 390; E-mail: nmacesic@vef.hr

ISSN 0372-5480
Printed in Croatia

similar. On the basis of the results of this study, comparing SCC and ZMT in mastitis diagnosis, ZMT is a reliable diagnostic method for use in field conditions.

Key words: Zagreb mastitis test, mastitis, somatic cell count, cow

Introduction

Mastitis is a multifactorial disease and is usually the result of interaction between microorganisms, the host and the environment. Mastitis occurs frequently in dairy cows, and causes significant economic losses that are manifested in reduced production and low milk quality (GRAČNER et al., 2006; BAČIĆ, 2009). According to epidemiological data, mastitis is one of the most important factors for culling. In Switzerland, 13% cows are culled from the herd due to mastitis (AEBERHARD et al., 1997). PAVLAK et al. (2008) analyzed 118 papers published in the Current Contents database in the period from 1997 to 2007 describing the prevalence and incidence of mastitis in the world, and reported that the prevalence of subclinical mastitis ranged from 6.1% in Switzerland to 70% in Brazil.

It is well-known that there is a high risk of developing subclinical mastitis in the early lactation period and a high percentage of intramammary infections (IMI) in the postpartum period (DE VLIEGHER et al., 2005; BAČIĆ, 2009). Due to the significant economic losses it is important to prevent and diagnose mastitis as early as possible (GRAČNER et al., 2006; MAĆEŠIĆ et al., 2012).

The status of udder infection may be expressed as subclinical or clinical mastitis. Clinical mastitis is characterised by visible changes to the milk with the appearance of flakes or blobs. Udder edema, redness and pain may be present (TURK et al., 2012). Subclinical mastitis has been defined as inflammation without visible signs on the udder (KELLY, 2002). Subclinical mastitis implies inflammation within the udder, but not necessarily infection. Subclinical mastitis is usually characterized by a high somatic cell count (SCC), a 15% to 45% drop in milk production, in the absence of visible changes to the milk or udder (SCHUKKEN et al., 1995; RODRIGUEZ-ZAS et al., 2000). The accurate diagnosis of IMI requires the isolation of pathogenic bacteria from milk samples. Bacteriology testing is usually time-consuming and quite expensive. SCC represents the best parameter for determining milk quality and udder infection in bovines (PYÖRÄLÄ, 2003). A threshold of $<2\times10^5$ cells/mL is considered to be the most practical value used to define a mammary quarter as healthy (RUEGG and PANTOJA, 2013). Factors associated with increased SCC in milk are: udder infection, age, lactation stage, season, stress and herd management (BARKEMA et al., 1999; DOBRANIĆ et al., 2007).

The California mastitis test (CMT), first described and used in 1957 (SCHALM and NOORLANDER, 1957), has been accepted as a quick, simple test to check SCC from individual quarters or composite milk (SANFORD et al., 2006). The sensitivity and specificity of CMT reported in the literature are differ among authors (PYÖRÄLÄ, 2003).

The Zagreb mastitis test (ZMT) is a field test intended for the identification of cows and quarters with abnormal udder secretion. Depending on the number of cells, the visible change in the mixture varies from no reaction (up to $3*10^5$ cell/mL) to the formation of a gel with the consistency of egg white. Udder quarters giving a positive reaction should be submitted for microbiological examination (BENIĆ, 2005).

The research objective was to evaluate the ZMT as a screening test for diagnosis of subclinical mastitis in dairy cows as well as to determine the prevalence of udder infection on dairy farms. According to the Regulations on the quality of fresh raw milk (NN 102/2000), a value of 4×10^5 cell/mL was accepted as the SCC upper limit for good quality milk.

Materials and methods

Animals. A total of 389 dairy cows and 1549 quarters from 6 dairy farms were included in the research. The farms are located in the Varaždin country in the period from 2011 to 2013. The cows are Simmental, Holstein-friesian or their crosses. The cows were kept in closed stalls, in small herds or free in larger farms. At all the visited farms, the cows were milked twice daily. Larger farms with a free stall system had milking parlors, while at smaller farms, milking was performed at standing places using milking machines. Udder preparation was preformed by disinfecting udder teats and drying with clean paper towels or individual cloths.

Sampling. Samples for examination were taken before the evening milking. Teat ends were disinfected with cotton swabs soaked in 70% ethanol. The first few streams of milk were discarded. Approximately 10 mL of milk from each udder quarter was taken in sterile tubes and kept at +4 ºC during transport, and analyzed within 12 hours after sampling. Microbiological examination was carried out according to the method recommended by NMC (1999).

Each sample was examined using the Zagreb mastitis test (ZMT, Croatian Veterinary Institute, Zagreb, Croatia). ZMT is a field test intended for identification of cows and quarters with abnormal udder secretion. It contains alkyl-aryl sulphonate which destroys the cell membrane and induces DNA polymerization. Hence the intensity of the reaction in the mixture of equal aliquots of milk and reagent, i.e. the consistency of the mixture, serves as an approximation for somatic cell count in the milk sample. Values of physical and chemical reactions are negative (up to 3×10^5 cell/mL), one cross (3×10^5 to 5×10^5 cells/mL), two crosses (5×10^5 to 20×10^5 cells/mL) or three crosses (20×10^5 - 150×10^5 cells/mL) (BENIĆ, 2005).

The data obtained were related to the milk samples' bacteriological findings, SCC and Zagreb mastitis test results for each quarter. The sensitivity and specificity of the SCC and the mastitis test were determined according to the results of the bacteriological findings from milk that was used as the "gold standard". In the mastitis test validity assessment, a negative mastitis test value was taken as a negative result and all other obtained values of the mastitis test were noted as positive (+, ++, +++).

For the evaluation of SCC as a screening test, the cut-off from 4×10^5 cell/mL in the milk samples was taken as negative, according to the Regulations on the quality of fresh raw milk (NN 102/2000), and a value of 4×10^5 cell/mL was accepted as the SCC upper limit for good quality milk. Milk samples were analyzed for SCC with a Fossomatic cell counter.

Statistical analysis. The agreement between SCC and ZMT was analyzed using the Kappa test. Values of 0.4 to 0.6 are interpreted as medium compatibility tests, while higher values indicate good and excellent compatibility tests (THRUSFIELD, 2007). For assessment of the validity of the SCC and mastitis test diagnostic procedures as well as the Kappa value assessment, the epidemiological program Win Episcope 2.0 was used.

Results

Evaluation of the mastitis test as a screening test for subclinical mastitis. Evaluation of ZMT validity as a screening test for subclinical mastitis was made based on mastitis test results and milk bacteriological findings from 1549 quarters.

Table 1. Results of bacteriological findings and ZMT (n = 1549 quarters)

Test	Bacteriological findings (positive)	Bacteriological findings (negative)	Total
Zagreb mastitis test (positive)	736	243	979
Zagreb mastitis test (negative)	24	546	570
Total	760	789	1549

Results of ZMT and bacteriological findings for each quarter milk sample are shown in Table 1. According to the data shown in Table 2, the test has high sensitivity (97%, 95% confidence interval, 96-98%), low specificity (69%, 95% confidence interval, 66% - 71%) and high negative predictive values (96%, 95% confidence interval, 94% - 97%).

Table 2. Assessment of the sensitivity and specificity of Zagreb mastitis test (n = 1549 quarters)

	%	*CI 95% -	*CI 95% +
Sensitivity	96.84	95.59	98.09
Specificity	69.20	65.98	72.42
True prevalence	49.06	28.49	51.55
Apparent prevalence	63.20	46.57	65.60
Positive predictive values	75.17	77.24	77.89
Negative predictive values	95.79	94.14	97.44

*CI 95% - = Lower Confidence Interval limit; CI 95% + = Upper Confidence Interval limit

Assessment of ZMT reliability as a screening test for subclinical mastitis was determined on the basis of mastitis test results and bacteriological findings from 1493 quarters. Results of bacteriological findings and SCC threshold of 4×10^5 cell/mL are shown in Table 3.

Table 3. Results of bacteriological findings and SCC threshold of 4×10^5 cell/mL

Test	Bacteriological findings (positive)	Bacteriological findings (negative)	Total
SCC $>4\times10^5$ cell/mL	654	98	752
SCC $<4\times10^5$ cell/mL	86	655	741
Total	740	753	1493

Evaluation of somatic cell count as a screening test for subclinical mastitis. According to the data in Table 3, SCC parameters, including sensitivity, specificity, predictive values and true prevalence as well as apparent prevalence, were calculated. All the parameters are shown in Table 4.

Table 4. Assessment of SCC for threshold of 4×10^5 cell/mL in the subclinical mastitis evaluation

	%	*CI 95% -	*CI 95% +
Sensitivity	88.38	86.07	90.69
Specificity	86.99	84.57	89.39
True prevalence	49.57	47.02	52.10
Apparent prevalence	50.37	47.83	52.51
Positive predictive values	86.97	84.56	89.37
Negative predictive values	88.39	86.09	90.70

*CI 95% - = Lower Confidence Interval limit; CI 95% + = Upper Confidence Interval limit

According to the data from Table 4, sensitivity (88%, 95% confidence rate, 86% - 91%) and specificity (87%, 95% confidence rate 84% - 89%) are equal, as well as positive and negative predictive values. The values of true prevalence (49.6%, 95% confidence rate 47% - 52%) and test obtained prevalence (50.3%, 95% confidence rate 48% - 52%) are equal.

Zagreb mastitis test and somatic cell count overlap evaluation. According to the data from Table 5, the Kappa test value was determined. The Kappa test for ZMT and SCC was 75.3% (0.753) indicating good test overlap and their correlation.

Table 5. Results of somatic cell count and ZMT

Test	SCC >4×10^5 cell/mL (positive)	SCC <4×10^5 cell/mL (negative)	Total
Zagreb mastitis test (positive)	738	185	923
Zagreb mastitis test (negative)	0	570	570
Total	738	755	1493

Discussion

The production of high quality milk is a requirement to sustain a profitable dairy industry. SCC has been used for a long time as a tool for measuring milk quality (DOHOO and LESLIE, 1991) and as a diagnostic tool for subclinical mastitis detection. For subclinical mastitis diagnostics there are many methods and tests, but the simplest and most practically reliable is the California mastitis test (CMT) (DINGWELL et al., 2003; BENIĆ, 2005; DE VLIEGHER et al., 2005; DJURICIC et al., 2014) and similar tests such as the ZMT. CMT represents a suitable test for subclinical mastitis herd inspection in 84% of cases (PYÖRÄLÄ, 2003).

Using a cut-off for ZMT with scores of ≥1 as positive, 63.20% (CI 95% 60.80% - 65.60%) quarter milk samples were estimated as being positive for mastitis. This cut-off had high sensitivity (96.84%, CI 95% 95.60% - 98.06%) and negative predictive value (95.79%, CI 95% 94.14% - 97.44%), however specificity (69.20%, CI 95%, 65.98% - 72.74%) and positive predictive value (75.17%, CI 95%, 72.47% - 77.86%) were low. The sensitivity and specificity of CMT reported in the literature is variable (PYÖRÄLÄ, 2003). For example, at 3 days, SARGEANT et al. (2001) found that the sensitivity and specificity that were able to identify any IMI in milk at quarter level were 57% and 56%, respectively. On the other hand, VIJAYA REDDY et al. (1998) reported sensitivity of 71% and specificity of 75%. Due to the great differences in sensitivity and specificity it can be expected that a higher prevalence is obtained using a mastitis test than the true prevalence (Table 1). In our study, the true prevalence was 49%, while the prevalence obtained with the mastitis test was 63%, which means that there were 14% false positive results. In assessing the value of positive and negative test results on an individual level, it was estimated with 96% probability that a quarter with a negative mastitis test is indeed uninfected (Table 1.). The relationship between the CMT and bacteriological findings ranged from 70% - 86% depending on the pathogen (SANFORD et al., 2006; BASTAN et al., 2008). VARATANOVIĆ et al. (2010) published that 63.3% of the examined cows had a positive reaction to the ZMT. In their research, the percentage of correspondence between CMT and bacteriological findings was 55.7%. MAĆEŠIĆ et al. (2013) pointed out that number of ZMT false positive quarters depends on the lactation stage. The number of false positive CMT results increased in later lactation stages.

SCC thresholds are often used to predict intramammary infection (IMI) at either the quarter or cow level. However, it has been proposed that quarters having a cell count of 2×10^5 cell/mL and whole cow milk cell count of 4×10^5 cell/mL indicate mastitis (SHARMA et al., 2011). For the evaluation of SCC as a screening test, the cut-off from 4×10^5 cell/mL in the milk samples has been taken as negative, according to the Regulations on the quality of fresh raw milk (NN 102/2000), and the value of 4×10^5 cell/mL is accepted as the SCC upper limit for good quality milk. Bulk tank SCC (BTSCC) values are routinely used to define the national and international regulatory standards in hygienic milk production (DOBRANIĆ et al., 2008). The national standards for BTSCC vary from $<4\times10^5$ cell/mL (EU, Australia, New Zealand and Canada) to $<10\times10^5$ cell/mL (Brazil) (MAĆEŠIĆ et al., 2013). In our study, assessment of SCC as a screening procedure for the threshold of 4×10^5 cell/mL had 88% sensitivity and 86% specificity (Table 4), while the prevalence and true prevalence determined on the basis of the SCC results were 50% and 49% respectively (Table 4). GONZALEZ RODRIGUEZ and CARMENES (1996) reported that prevalence was 43% for a threshold of 3×10^5 cell/mL and the true prevalence was 39.2%, evaluating SCC in the diagnosis of mastitis. DOHOO and LESLIE (1991) evaluated sensitivity and specificity using several SCC thresholds (2×10^5 - 3×10^5 cell/mL). The reported sensitivities and specificity range from 73 to 89% with corresponding specificities of 75-85%. Sensitivity and specificity are improved when only major pathogens are considered (SARGEANT et al., 2001; DINGWELL et al., 2003). ¸

In this study, we compared the results of ZMT with those of SCC by means of the Kappa test. The accord between these two methods was found to be high (0.753). BARBOSA et al. (2002) reported that SCC and CMT are dependent and highly correlated for diagnosis of subclinical mastitis. In comparison to the ZMT, for estimation of prevalence, the SCC showed more accuracy. Since the ZMT has high sensitivity and high negative predictive values, it may be a good screening test to identify healthy animals in a herd.

We acknowledge that the bacterial culture of milk is not an adequate gold standard for validating ZMT or SCC in lactating cows. Bacteriological culture of single quarter milk samples of lactating cows is somewhat inefficient (BRADLEY et al., 2005). The numerators may be biased downward in our positive predictive value calculations and biased upward in our negative predictive calculations. Consequently, the predictive value calculation estimates could be biased downward and the negative predictive calculation estimates biased upward relative to the values that would be obtained if the bacterial culture had no false negative rates. The magnitude of bias in our estimated predictive values is not known and would be a function of the degree of inefficiency in the bacterial diagnosis protocol (ROY et al., 2009).

In conclusion, it is important to know how to define the limits for the tests that are read in continuous values, such as SCC. Sensitivity and specificity are changed by altering the limit value parameters. SCC was the most accurate test after microbiological examination, followed by ZMT. Taking into consideration that microbiological examination and SCC require adequate laboratory facilities, ZMT is a reliable diagnostic method for use in field conditions.

References

AEBERHARD, K., R. BRUCKMAIER, J. W. BLUM (1997): High-yielding dairy cows in Schwizerland. Agraforschung 4, 277-280.

BAČIĆ, G. (2009): Diagnostic and mastitis treatment in dairy cows (in Croatian), (Bačić, G., S. Vince, N. Maćešić, Eds.), Faculty of Veterinary Medicine, University of Zagreb, Zagreb, pp. 4-20.

BARBOSA, K. B., E. BENADETTI, S. C. RIBERO, E. C. GUIMARAES (2002): The relationship between somatic cell count (SCC) and result of the California mastitis test (CMT) to diagnose mastitis. Biosci. J. 18, 93-102.

BARKEMA, H. W., J. D. VAN DER PLOEG, Y. H. SCHUKKEN, T. J. G. M. LAM, G. BENEDICTUS, A. BRANDS (1999): Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. J. Dairy Sci. 82, 1655-1663.

BASTAN, A., C. KACAR, B. D. ACAR, M. SAHIN, M. CENGIZ (2008): Investigation of the incidence and diagnosis of subclinical mastitis in early lactation period cows. Turk. J. Vet. Anim. Sci. 32, 119-121.

BENIĆ, M. (2005): The frequency of mastitis before and after adoption of regulation on quality of fresh raw milk (in Croatian). Vet. stn. 36, 233-238.

BRADLEY, A. J., H. NEWTON, M. J. GREEN (2005): Use and interpretation of bacteriology in the diagnosis of bovine intramammary infection. Proceedings of the 4th IDF International Mastitis Conference. June 12-15, 2005, Maastricht, Netherlands, pp. 409-415.

DE VLIEGHER, S., H. W. BARKEMA, H. STRYHN, G. OPSOMER, A. DE KRUIF (2005): Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. J. Dairy Sci. 88, 938-947.

DINGWELL, R. T., K. E. LESLIE, Y. H. SCHUKKEN, J. M. SARGEANT, L. L. TIMMS (2003): Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can. Vet. J. 44, 413-416.

DJURICIC, D., M. SAMARDZIJA, J. GRIZELJ, T. DOBRANIC (2014): Effet du traitement intramammaire des mammites subcliniques pendant la lactation en élevages bovins laitiers au nord-ouest de la Croatie. Ann. Méd. Vét. 159, 121-125.

DOBRANIĆ, V., B. NJARI, M. SAMARDŽIJA, A. SELEC, B. MIOKOVIĆ, V. MEDVID (2007): Saisonbedingte Änderungen der mikrobiologischen Milchflora unter besonderem Berücksichtigung auf von Pilzen. Tierärztl. Umschau 62, 300-309.

DOBRANIĆ, V., B. NJARI, M. SAMARDŽIJA**,** B. MIOKOVIĆ, R. RESANOVIĆ (2008): The influence of the season on the chemical composition and the somatic cell count of bulk tank cow's milk. Vet. arhiv 78, 235-242.

DOHOO, I. R., K. E. LESLIE (1991): Evaluation of changes in somatic cell counts as indicators of new intra-mammary infections. Prev. Vet. Med. 10, 225-237.

GONZALEZ RODRIGUEZ, M. C., P. CARMENES (1996): Evaluation of California mastitis test as a discriminant method to detect subclinical mastitis in ewes. Small Rumin. Res. 21, 245-250.

GRAČNER, D., LJ. BEDRICA, M. CERGOLJ, I. HARAPIN, M. SAMARDŽIJA, G. GREGURIĆ GRAČNER, D. ŽUBČIĆ, J. REŠETIĆ, M. FURY (2006): Haptoglobinspiegel in Blut und Milch von Kühen mit einer Staphylokokkenmastitis. Tierärtzl. Umschau 61, 636-641.

KELLY, A. L. (2002): Test methods and standards. Encyclopedia of Dairy Sciences. Academic Press. 3, pp. 1995-2002.

MAĆEŠIĆ, N., M. CERGOLJ, G. BAČIĆ, B. RAŠIĆ, D. CVITKOVIĆ, T. KARADJOLE, M. LOJKIĆ, N. PRVANOVIĆ BABIĆ, M. BENIĆ, M. PAVLAK (2013): Assessment of Zagreb mastitis test and somatic cells in diagnosis subclinical mastitis caused by bacteria *Staphylococcus aureus*. Proceedings of XIII Middle European Buiatric's Congress. June 5-8, 2013, Beograd, Serbia, pp. 442-448.

MAĆEŠIĆ, N., T. KARADJOLE, G. BAČIĆ, M. BENIĆ, M. KARADJOLE, S. VINCE, M. LIPAR, M. CERGOLJ (2012): Aetiology and prevalence of bovine intramammary infection at drying off. Vet. arhiv 82, 125-131.

NATIONAL MASTITIS COUNCIL (1999): Laboratory handbook on bovine mastitis. National mastitis council Inc., Madison, WI 53704-6797, USA. Revised edition.

PAVLAK, M., M. BENIĆ, D. CVITKOVIĆ, M. TADIĆ (2008): Epidemiological data of intramammary infection in cattle - a quantitative analysis of published data. Proceedings of the XVI. Congress of the Mediterranean Federation for Health and Production of Ruminants, 22-26 April. Zadar, Croatia, pp. 97-112.

PYÖRÄLÄ, S. (2003): Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 34, 565-578.

RODRIGUEZ-ZAS, S. L., D. GIANOLA, G. E. SHOOK (2000): Evaluation of models for somatic cell score lactation patterns in Holsteins. Livest. Prod. Sci. 67, 19-30.

ROY, J. P., D. DU TREMBLAY, L. DESCOTEAUX, S. MESSIER, D. SCHOLL, E. BOUCHARD (2009): Evaluation of the California mastitis test as a precalving treatment selection tool for Holstein heifers. Vet. Microbiol. 134, 136-142.

RUEGG, P. L., J. C. F. PANTOJA (2013): Understanding and using somatic cell counts to improve milk quality. Irish J. Agr. Food Res. 52, 101-117.

SANFORD, C. J., G. P. KEEFE, J. SANCHEZ, R. T. DINGWELL, H. W. BARKEMA, K. E. LESLIE, I. R. DOHOO (2006): Test characteristics from latent-ciass models of the California Mastitis test. Prev. Vet. Med. 77, 96-108.

SARGEANT, J. M., K. E. LESLIE, J. E. SHIRLEY, B. J. PULKRABEK, G. H. LIM (2001): Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation. J. Dairy Sci. 84, 2018-2024.

SCHALM, O., D. NOORLANDER (1957): Experiments and observations leading to the development of California mastitis test. J. Am. Vet. Med. Assoc. 130, 199-204.

SCHUKKEN, Y. H., T. J. LAM, M. NIELSON, H. HOGEVEEN, H. W. BARKEMA, F. J. GROMMERS (1995): Subclinical and clinical mastitis on dairy farms in The Netherlands: epidemiological developments. Tijdschr. Diergeneeskd. 120, 208-213.

SHARMA, N., N. K. SINGH, M. S. BHADWAL (2011): Relationship of somatic cell count and mastitis: An overview. Asian-Aust. J. Anim. Sci. 24, 429-438.

THRUSFIELD, M. (2007): Diagnostic testing. In: Veterinary Epidemiology. 3rd ed. (Thrusfield, M., Ed.). Oxford, Blackwell Science. pp. 305-29.

TURK, R., C. PIRAS, M. KOVAČIĆ, M. SAMARDŽIJA, H. AHMED, M. DE CANIO, A. URBANI, Z. FLEGAR MEŠTRIĆ, A. SOGGIU, L. BONIZZI, P. RONCADA (2012): Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. J. Proteomics 75, 4412-4428.

VARATANOVIĆ, N., M. PODŽO, T. MUTEVELIĆ, K. PODŽO, B. ČENGIĆ, A. HODŽIĆ, E. HODŽIĆ (2010): Use of California mastitis test, somatic cells count and bacteriological findings in diagnostics of subclinical mastitis. Biotechnol. Anim. Husb. 26, 65-74.

VIJAYA REDDY, L., P. C. CHOUDHURI, P. A. HAMZA (1998): Sensitivity, specificity and predictive values of various indirect tests in the diagnosis of subclinical mastitis. Indian Vet. J. 75, 1004-1005.

Received: 19 May 2015
Accepted: 22 March 2016

MAĆEŠIĆ, N., G. BAČIĆ, K. BOŽIČEVIĆ, M. BENIĆ, T. KARADJOLE, N. PRVANOVIĆ BABIĆ, M. LOJKIĆ, M. EFENDIĆ, I. BAČIĆ, M. PAVLAK: Učinkovitost zagrebačkog mastitis testa u otkrivanju supkliničkog mastitisa krava. Vet. arhiv 86, 475-485, 2016.

SAŽETAK

Cilj je ovog rada bio procijeniti pouzdanost zagrebačkog mastitis testa i broja somatskih stanica kao probirnih testova u dijagnostici mastitisa. Istraživanje je provedeno u okviru nadzora supkliničkog mastitisa na području Varaždinske županije. U istraživanje je bilo uključeno 389 mliječnih krava simentalske pasmine, odnosno ukupno 1549 uzoraka mlijeka. Uzorci mlijeka za mikrobiološku pretragu i mastitis test uzeti su u sterilne plastične epruvete prije redovite dnevne mužnje mlijeka. Bakteriološkom pretragom dobili smo 760

(49,06%) pozitivnih uzoraka. *Staphylococcus aureus* izdvojen je iz 388 (51,05%) uzoraka dok je *Streptococcus* spp. izdvojen iz 84 (11,05%) uzorka pozitivnih četvrti. Procjenom zagrebačkog mastitis testa kao probirnog dijagnostičkog postupka ustanovljeno je da ima visoku osjetljivost (96,84%, CI 95% 95,60% - 98,06%) ali nisku specifičnost (69,20%, CI 95% 65,98% - 72,74%) te visoke negativne prediktivne vrijednosti (95,79%, CI 95% 94,14% - 97,44%). Za određivanje broja somatskih stanica kao dijagnostičkog postupka uspoređena je granična vrijednost od 4×10^5 st/mL. Osjetljivost tog testa iznosila je 88,37% (CI 95%, 86,06% -90,68%), a specifičnost 86,98% CI 95% 84,57% - 89,38%) dok su prediktivne vrijednosti bile podjednake. Rezultati ukazuju da su oba testa ovisno o svojim karakteristikama dobri pokazatelji mastitisa kod krava.

Ključne riječi: zagrebački mastitis test, mastitis, broj somatskih stanica, krava