FARM RECORDS IN INVESTIGATION OF EPIDEMIOLOGY, SYMPTOMATOLOGY AND CAUSES OF CLINICAL MASTITIS IN A DAIRY FARM

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Abstract: Mastitis is one of the most important diseases in dairy cow farms and one of the most common cause for antibiotic treatment. Aims of this study were: to investigate frequency and trends of clinical mastitis in cows on a large dairy farm, describe clinical characteristics of mastitis and investigate causative infectious agents in selected cases alongside antimicrobial resistance.
In our study we used farm records for clinical mastitis recorded for period 2016 and 2017. We also used results of the regular on farm testing of the somatic cell count for 2017. Samples of milk from all clinical mastitis cases were taken during November and December 2017 in order to investigate causative agents and their antimicrobial resistance.
Occurrence of clinical mastitis was 205 cases (47.7%) in 2017 compared to 93 cases (29.7%) recorded in 2016. In 2017 reoccurrence of clinical mastitis in same animal was recorded for 93 cows (45.4%). In 2016 reoccurrence of clinical mastitis in same animal was recoded for 49 cows (29.7%). Average course of clinical mastitis in 2016 was 3 days, while in 2017 4.5 days (continuous days of recording a case in farm records). Somatic cell count in more than half of tested animals was higher than 200.000 SC/ml according to the measurements from February and July 2017 (number of cows tested 236 and 169, respectively). Out of 23 milk samples, 20 had bacteriological growth. In 9 samples we identified *S.aureus*, in 6 streptococcus spp., in 4 coagulase negative staphylococci (CNS) and one sample contained *E.coli*. Most common resistance was found for lincomycin-spectinomycin (100%) gentamicin (92%), followed by cefquinome (65%), linkomycin (53%) and erythromycin (47%). Isolates of *S.aureus* were resistant on the largest number of investigated antibiotics.

Key words*: clinical mastitis, trends, etiology, antimicrobial resistance

Introduction

Improvement of production technologies and hygiene on farms accompanied with programs for control of important infectious diseases causing productions losses and public health threats (i.e. brucellosis, tuberculosis) had led to eradication of many of these diseases particularly in developed countries. Simultaneously, other diseases became important especially in intensive production systems, commonly called breeding diseases such as lameness, reproductive and metabolic disorders and mastitis (*Zwart, 1997*).

Mastitis in dairy animals is recognized worldwide as one of the most expensive diseases in modern farm production (*Seegers, 2003*). In addition to burden of infectious mastitis which causative agents have public health importance, mastitis related production losses are one of the most important limiting factors of dairy production in Bosnia and Herzegovina (BiH) (*Alagić, 2006*). Prevalence of either clinical or subclinical mastitis on farms in BiH can reach more than 50% (*Varatanović, 2010*). Most commonly about half of these cases are clinical mastitis, where most commonly isolated causative agents are *Staphylococcus aureus*, *Streptococcus agalactiae*, coagulase positive staphylococci, *Trueperellapyogenes*and coliforms (*Matarugić, 2009; Varatanović, 2009*).

Some bacteria causing mastitis in dairy cows can also cause different diseases in humans, however important public health aspect of mastitis in animals is linked with occurrence of residues in milk due to non-selective use of antimicrobials in treatment and control of this disease. Residues of antimicrobials in food can harm human health directly, but also lead to increase of the antimicrobial resistance in bacteria and potential for spread of theses resistant agents throughout the food chain, in addition to reducing effectiveness of mastitis treatment in animals (*Tenhagen, 2006*).

Aims of our study were: first to establish annual prevalence of clinical mastitis on large commercial farm, second to describe trends of mastitis occurrence and severity of clinical findings, third to investigate causes of clinical mastitis in some cases and fourth to investigate antimicrobial resistance of isolated agents.

Materials and methods

With consent and guarantee of private and business information protection, this study used following data from farm records of a commercial dairy farm:
- Records of the farm veterinary service for clinical mastitis cases in 2016 and
  2017, which contained date of symptoms onset/recognition, ear tag number for

diseased animal and number of affected quarters. Individual animals were kept in records for consecutive days until termination of symptoms and/or therapy,

- Results of the somatic cell count in milk of individual animals measured alternately in stables A and B throughout 2017 (quarterly).

Sampling of the milk from cows with clinical mastitis was done on same farms in order to establish causative agents and their antimicrobial resistance using disk-diffusion method (antibiogram). Laboratory investigations were done at Laboratory for bacteriology and mycology of the Institute of Veterinary faculty University of Sarajevo.

Data management, analysis and graphical representation of study results were done using Excel (Microsoft Office (r)).

Using farm records for clinical mastitis in 2016 and 2017 we created Excel data base containing 556 entries. By eliminating repeated entries of animals with same ear tag number in time frame shorter than 15 days since the same animal was firstly recorded as mastitis case, data base of prevalent cases of clinical mastitis was created containing 370 cases in two year period. Repeated record of the same animal (same ear tag number) longer than 15 days from last entry was considered to be repeated case of mastitis in same animal. Also based on prevalent cases data base we created data base containing new (incident) cases of mastitis on monthly basis, which contained 338 records (animals recorded as cases in previous month were not counted as new cases in following moth).Case definition was observation of clinical symptoms of mastitis and/or changes in milk found in one or more quarters. In addition to data on the frequency of clinical mastitis, we analyzed severity of clinical findings (number of affected quarters, duration of symptoms) on monthly and annual basis.

Results of the somatic cell count in milk of the individual animals were analyzed separately, since sampling was conducted only in clinically healthy animals. Since farm has two stables (A and B), SCC was conducted for animals in stable A in February and September 2017, and in July and November for animals in stable B. Results of the SCC were stratified in 4 categories (<200.000 SC/ml, 200.000 – 500.000 SC/ml, 500.000 – 1.000.000 SC/ml, >1.000.000 SC/ml), and expresses as proportion for each category with respect to overall animal tested in each occasion. By comparing SCC results with data bases of clinical mastitis we identified numbers of occurring cases in period one month before and after SCC testing.

Milk samples were taken from all animals with clinical mastitis registered during November and December 2017, before any treatment was administrated. Microbiological isolation of agents was done using standardized laboratory protocols (*Quinn, 2011*).

For establishing average of affected quarters in clinical mastitis cases on monthly and annual basis we calculated Mode (the most frequent observation), while for average length of mastitis we used the mean (arithmetic average). For

comparison of proportions (prevalence) we used chi-square test for homogeneity of proportion interpreted for level of statistical significance of 5% (α=0.05).

Results and Discussion

Comparing established occurrence of clinical mastitis in 2016 and in 2017 (Figure 1) we established higher frequency of cases in 2017 on monthly and annual basis. Annual prevalence for 2016 was 38.4%, while for 2017 47.7%. Difference between annual prevalence was found to be statistically significant ($\chi 2$=7,6, p value 0,00587).
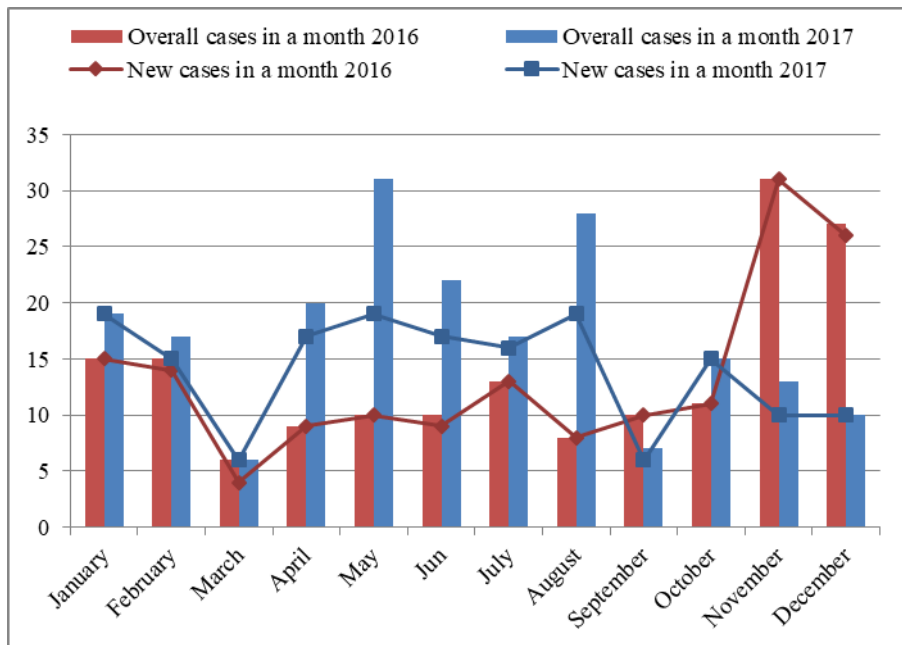

Figure 1: Cases of clinical mastitis (prevalent cases –bars, incident cases – lines) on monthly basis for 2016 (in red) and 2017 (in blue)

Our results also showed that in many animals mastitis is reoccurring within one or two year period (Figure 2). From 205 cases of clinical mastitis in 2017, 45.4% (93/205) were recurrent twice or more times in a same animal. In 2016 from overall 165cases, recurrence was 29.7% (49/165). For two year period (2016 and 2017), proportion of reoccurring cases was 49.5% (183/370).
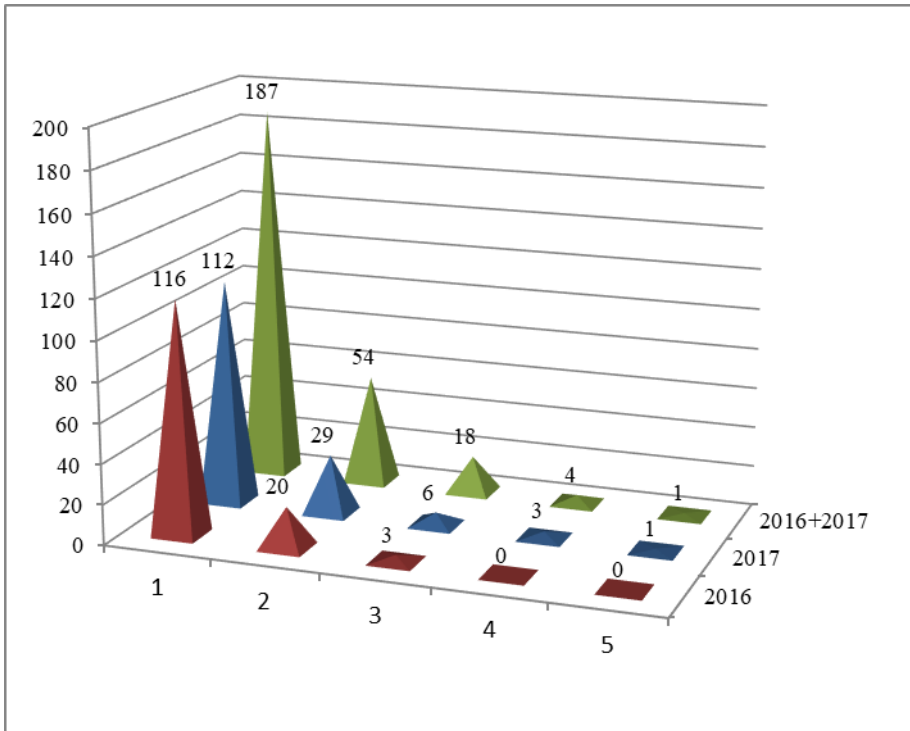
Figure 2: Repeating occurrence (x axes) of clinical mastitis in same animals within a year (2016 – in red, 2017 – in blue), and within two years (2016+2017 in green)

Higher frequency and reoccurrence of clinical mastitis in 2017 was accompanied with higher average number of affected quarters (Figure 3).
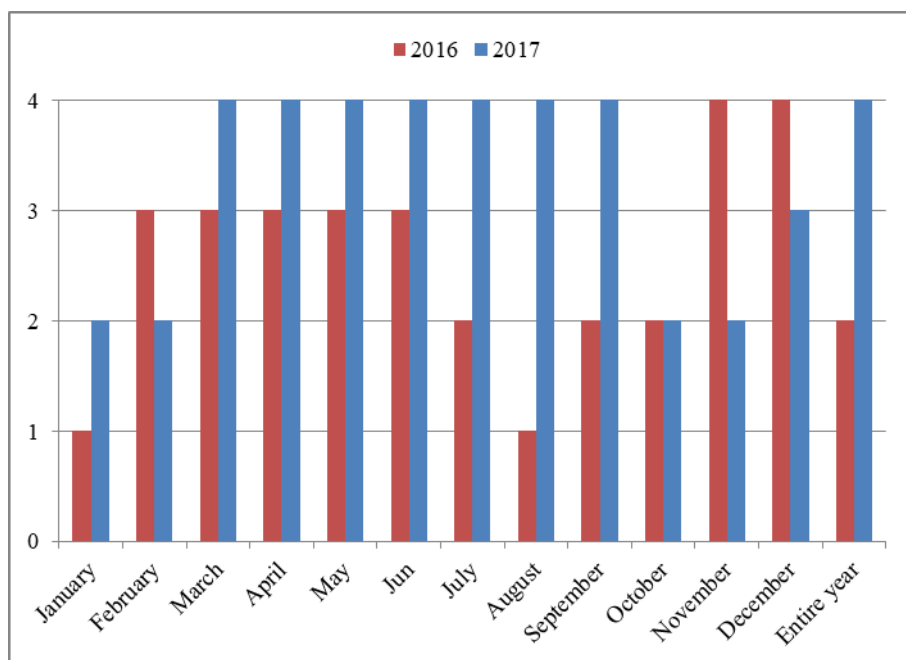
Figure 3: Average number of affected quarters in clinical mastitis cases (mode) on monthly and annual basis (2016 - in red, 2017 – in blue)

In most animals symptoms had disappeared after at most 3 days. Number of animals in which symptoms lasted between 8 and 15 days and over 15 days in 2016 was 12 and 7 respectively, and in 2017   17 and 15, respectively. Average duration on clinical mastitis symptoms in 2016 was 3 days (mean), while in 2017 4.5 days.

Results of the SCC in milk (Figure 4), shows that more than half of tested animals had >200.000 SC/ml according to measurement from February and July 2017, coinciding that in same months the largest number of clinical mastitis cases was observed (in comparison with other two months of SCC testing). In September and November 2017, proportion of animals with >200.00 SC/ml of milk was 34.4% and 32.1%, respectively whereas in same months 7 and 13 cases of mastitis was recorded respectively.
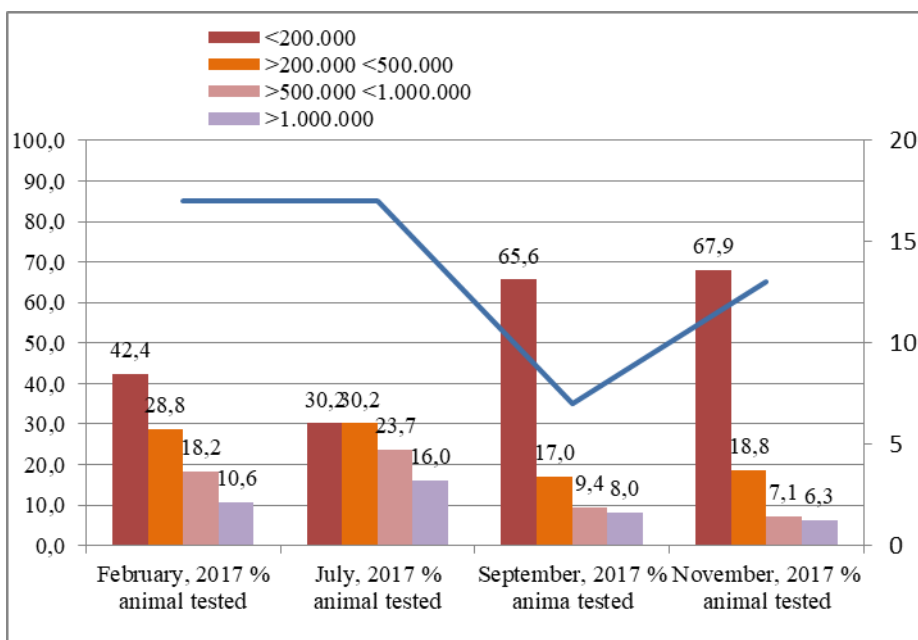
Figure 4: Results of the SCC in milk measured quarterly during 2017, stratified by categories (% (bars) - left axes, February – in red, July – in orange, September – in pink, November- in violet) shown with number of clinical mastitis cases in same months (blue line – right axes)

Because cases of subclinical mastitis recognized by increase of SC/ml commonly progress to clinical mastitis, as well as cases of chronic mastitis, table 1 shows number of clinical mastitis cases recorder one month before and 1 month after SCC testing stratified by given SCC categories. Progression of subclinical cases in clinical is particularly indicative for SCC testing in February (one month after SCC testing). Also for many cases of clinical mastitis recorded in January number of SCC measured in February remained high.

Table 1: Number of recorded clinical mastitis cases one month before and one month after measurement of SCC, stratified by SCC categories

SCC	Month of SCC testing							
	II 2017.		VII 2017.		IX 2017.		XI 2017.	
	-1mo.	+1mo.	-1mo.	+1mo.	-1mo.	+1mo.	-1mo.	+1mo.
<200.000	2	4	-	-	2	2	1	2
200.000-500.000	1	1	-	-	-	-	2	-
500.000-1.000.000	4	1	-	-	-	-	-	-
>1.000.000	4	3	-	12	-	-	-	1

Twenty milk samples, of total 23, had bacteriological growth. *S.aureus* was identified in 9 samples, *Streptococcus* spp. in 6, CNS in 4 samples and one sample contained *E.coli*. Table 2 contains results of testing for antimicrobial resistance in bacterial isolates.

Table 2: Results of the antimicrobial resistance testing or identified causative agent of clinical mastitis shown as proportion (%) of sensitive (S), moderately sensitive (I) and resistant isolates

	Overall isolates N=20			*S.aureus Isolates* N=9			CNS Isolates N=4			*Streptococcus* spp. Isolates N=6			*E.coly* isolate
Antibiotic	S	I	R	S	I	R	S	I	R	S	I	R	S/I/R
Amoxicillin/ clavulanic acid	75	5	20	78	-	22	50	-	50	83	-	17	S
Ampicillin/ sulbactam	75	25	-	78	22	-	50	50	-	100	-	-	I
Cefquinome	25	10	65	22	-	78	50	-	50	-	33	67	S
Ceftiofur	30	40	30	22	67	11	50	50	-	17	-	83	S
Cephalothin	100	-	-	100	-	-	100	-	-	100	-	-	ni*
Ciprofloxacin	50	15	35	67	-	33	75	-	25	-	50	50	S
Linkomycin	5	42	53	-	55	45	25	25	50	-	17	83	ni
Erythromycin	21	32	47	-	22	78	25	25	50	50	50	-	ni
Gentamicin	8	-	92	-	-	100	25	-	75	ni			ni
Marbofloxacin	100	-	-	100	-	-	100	-	-	ni			ni
Penicillin	65	-	35	67	-	33	50		50	67	-	33	S
Cefprozil	100	-	-	100	-	-	100	-	-	100	-	-	ni
Mastijet (neomicin, bacitracin, tetracicline)	95	-	5	100	-	-	100	-	-	100	-	-	- R
linkomicin-spectinomicin	-	-	100	-	-	100	-	-	100	100			ni

* not investigated

Mastitis in cows is inflammation of mammary gland, commonly caused by infection with microorganisms. Large number of microorganisms is recognized as causes of mastitis and many of them have direct or indirect public health importance (*Watts, 1988; Hameed, 2006*). Mastitis is also leading disease according to economic losses in dairy cow farms and most common cause for antibiotic treatment (*Seegers, 2003; Pol, 2007; Saini, 2012*).

Annual clinical mastitis prevalence established in this study (for 2016. 38.4%, and for 2017. 47.7%) coincides with results of similar earlier studies conducted in BiH (*Varatanović, 2010*). Comparative studies from other countries report range of established prevalence; from 21.5% in Ethiopia (*Workineh, 2002*), 26.4% in Germany (*Terhagen, 2006*), 31% in Finland (*Pitkälä, 2004*), up to 52.4% in Uruguay (*Gianneechini, 2002*). Clinical mastitis prevalence at dairy farm in BiH that we established falls into this range; however there is space for improvement

and reaching values of herd/farm prevalence established in developed countries. Further on, we recorded statistically significant increase of prevalence over two year period. During 2017 occurrence of clinical mastitis was higher in period from April to August compared to the rest of the year (118:87 case ratio), while in 2016 this ratio was 50 cases in period April-August compared to115 cases in rest of the year. Increased occurrence of clinical mastitis in warmer season of the year is related to better conditions for growth and spread of environmental mastitis pathogens (*Riekerink, 2007*). Therefore difference in clinical mastitis occurrence and seasonality between 2016 and 2017 can be explained by different etiology. This is also supported by established difference in clinical manifestation of mastitis in each year (average number of affected quarters and length of disease). Clinical mastitis caused by environmental bacteria is most commonly has shorter course and usually affects only one quarter *(Erskine, 2016)*. In samples of milk we investigated in two final months of 2017 leading cause of clinical mastitis was *S.aureus*, which is considered infectious mastitis pathogen, manifested with more severe clinical manifestation. Norwegian study conducted in 1997 established that leading causative agent of mastitis found in milk samples collected during late fall and early winter is *S.aureus* followed by *A.pyiogenes* (Waage, 1999). Same study found more *E.coli* in samples collected during summer months.

Early detection of mastitis on farms is accomplished by monitoring of the SCC, especially for subclinical mastitis. One month after SCC measurement on investigated farm conducted in February and July 2017 subclinical mastitis (SCC>200.000 SC/ml) evolved in clinical in 5 and 12 animals respectively. This shows that monitoring and control programs for subclinical mastitis are important preventive measure against clinical mastitis.

By microbiological cultivation of milk samples we established following causative agents; S.*aureus*, CNS, streptococci and *E.coli*. Study conducted in France in 2007 and 2008 found that most common causative agents of mastitis in dairy cows were *S.uberis* (22.1%), *E.coli* (16%) and coagulase positive staphylococci (15.8%) (*Bortel, 2010*). On the other hand German study (2001/2002) indicated that leading causes of mastitis in cattle was CNS, followed by *Corynebacterium bovis* and *S.aureus* (*Terhagen, 2006*). In Finland most commonly isolated bacteria associated with mastitis were CNS and *S.aureus* (*Pitkälä, 2004*). Obviously primary causes of mastitis in developed countries are environmental mastitis pathogens, indicating success of mastitis control programs primarily aimed against infectious pathogens such as *S.aureus* are *S.agalactiae (Hillerton, 2005).* However, infectious mastitis pathogens are still major issue on dairy farms in developing countries, as shown by our study as well (*Gianneechini, 2002; Workinch, 2002*). Together with shift in importance of different mastitis pathogens, increased occurrence of resistance in *S.aureus* and CNS isolates is observed, particularly for beta lactam antibiotics (*Myllys; 1998*).

Primary importance of increased resistance in these bacteria is resulting risk for human health, however simultaneously this impairs efficiency and options for mastitis treatment. Commonly it is very difficult to obtain antimicrobial usage data (type of antibiotic used, dosage, treatment regimen), especially if farmers themselves without consulting veterinarians are able to acquire and apply antibiotic treatment. Studies report proportion of resistant isolates on penicillin is for *S.aureus* from 17% to 52% (compared to overall number of *S.aureus* isolates from milk of mastitis cases), and for CNS from 30,7% to 40% (*Pitkälä, 2004; Terhagen, 2006; Botrel, 2010)*. This concurs with our results, however same research report much less proportion of resistant isolates of these bacteria to erythromycin, gentamycin and lincomycin. Moreover established resistance of our *S.aureus* and CNS isolates to antibiotics not used in food animals or in some cases contraindicated for treatment of mastitis indicates former unselective usage. Penicillin resistant S.*aureus* isolates are found in only 4% in Norway where legislation prescribes that only veterinarians make decision and administer antibiotic treatment of animals, while in countries where this is legally enabled to farmers as well, proportion of resistant strains is much higher up to a point when this antibiotic (most commonly used in mastitis treatment) is uttermost ineffective (*Oliver, 2012*).

Mastitis in dairy cattle is therefore complex disease occurring as a result of interaction of many factors related to animal itself, causative pathogen and the environment. Earlier epidemiological studies have led to establishment and widespread of simple mastitis prevention measures such as tit disinfection after milking and dry cow treatment (*Watts, 1988*). In order to reduce occurrence of mastitis in our farms it is recommended to introduce and fully implement these standard preventive measures alongside ensuring early detection of mastitis (using SCC and California mastitis test), isolation of diseased animals, culling of repeated cases, microbiological monitoring and testing for antimicrobial resistance before treatment is administered (*Workinech, 2002*). Keeping farm records and using them to improve effectiveness of decisions regarding treatment options for individual animals represents a basis for sound and responsible dairy production.

Conclusion

Annual clinical mastitis prevalence established in this study corresponds to same figures found in the country earlier, however it could be reduced to the levels recorded in developed countries. Different seasonality, clinical course and severity compared between 2016 and 2017 indicate different etiology of disease. Comparison of SCC measurements and occurrence of clinical mastitis confirms that this is an important tool in recognizing subclinical mastitis but also chronic (reoccurring) mastitis cases. We established following causative agents; S.*aureus*,

CNS, streptococci and *E.coli*. Antibiotic resistance results from our isolates, concurs other research, however there was much less proportion of resistant isolates of isolated bacteria to erythromycin, gentamycin and lincomycin. Established resistance of *S.aureus* and CNS isolates to antibiotics not used/ contraindicated in food animals indicates former unselective usage.

Farmska evidencija za istraživanje epidemiologije, simptomatologije i uzročnika kliničkog mastitisa na farmi mlečnih krava

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Rezime

Mastitis je jedna od najvažnijih bolesti na farmama mlečnih krava i predstavlja jedan od najčešćih povoda primene antibiotika. Ciljevi ovog istraživanja bili su: istražiti učestalost i trendove kliničkog mastitisa krava na velikoj komercijalnoj farmi, opisati kliničke karakteristike i odrediti infektivne uzročnike u određenom broju uzoraka mleka, kao i njihovu antimikrobnu rezistenciju.

U našem istraživanju smo koristili evidenciju farme o kliničkom mastitisu za period 2016. i 2017. Godina. U istraživanju su korišćeni rezultati redovnog testiranja broja somatskih ćelija u mleku iz 2017. godine. Uzorci mleka od svih krava kod kojih je ustanovljen klinički mastitis tokom novembra i decembra 2017. godine su mikrobiološki ispitani i određena je antimikrobna rezistencija dobijenih bakterijskih izolata.

Ustanovili smo da je broj krava sa kliničkim mastitisom iznosio 205 (47,7%) u 2017. godini, a broj krava sa kliničkim mastitisom u 2016. godini bio je 165 (38,4%). Pojava kliničkog mastitisa bila je veća u 2017. godini po mesecima i ukupno u odnosu na 2016. godinu. U 2017. godini ponovljeni slučajevi kliničkog mastitisa su iznosili 45,4% (93/205). U 2016 godini ponovljeni slučajevi kliničkog matitisa su iznosili 29,7% (49/165). Prosečno trajanje kliničkog mastitisa u 2016. godini bilo je 3 dana, a u 2017. godini 4,5 dana (broj dana u kontinuitetu u evidenciji). Broj somatskih ćelija u mleku kod više od polovine testiranih životinja u februaru i julu 2017. godine bio je veći od 200.000 SC/ml (testirano 236 odnosno 169 krava). Od 23 uzorka mleka, 20 je imalo bakteriološki rast. U 9 uzoraka ustanovljena je *S.aureus*, u 6 streptococcus spp., u 4 koagulaza negativne stafilokoke i u jednom *E.coli*. Kod svih bakterijskih izolata najraširenija je rezistencija na lincomycin-spectinomycin (100%) gentamicin (92%), koje slede

cefquinome (65%), linkomycin (53%) i erythromycin (47%). Izolati *S.aureus* su bili rezistentni na najveći broj ispitanih antibiotika.

Ključne reči: klinički mastitis, trendovi, etiologija, antimikrobna rezistencija

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