Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2011, 2 (5) :552-562
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Assessment of the effect of homoeopathic nosodes in subclinical bovine mastitis

Mehdi Kiarazm*[1], Parviz Tajik[2] and Hamid Ghasemzadeh Nava[2]

[1]Department of Gynecology and Obstetrics, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

---

ABSTRACT

The aim of the present Study was to evaluate the effects of homeopathic medication containing two nosodes, staphylococcus aureus and streptococcus dysgalactiae in the treatment of sub-clinical mastitis.Three hundreds dairy cows were selected during the lactation period from one herd with a high somatic cell count (400000 ≤SCC≤1000000 cells/ml) with no signs of clinical mastitis. California mastitis tests (CMT), somatic cell count (SCC) and bacteriological culture (BC) were performed on each sample. All of the cows were assigned in a blind, randomized study, divided into two groups, treatment homeopathic nosodes group (160 cows) and placebo control group (140 cows), with a 5 ml daily amount for a period of 5 days. After treatment initiation, milk samples were taken from each group to determine the SCC and conduct a bacteriological culture. Data from the study was used to compare the two groups of cows on day 0, day 21 and day 28. After treatment, the SCC was significantly lower in the treated group compared with the control group on day 21 and 28, and a significant difference was seen between the BC results in the two groups. The results of the present study showed that the use of a homeopathic nosode for sub-clinical mastitis during the lactation period had a significant effect on treatment and the decreasing incidence of this disease. On the other hand, as there are no residues in the milk, and due to the lower costs involved, it is more economical.

***Key words**: sub-clinical mastitis, somatic cell count, homeopathy, staphylococcus aureus, streptococcus dysgalactiae.*

INTRODUCTION

In spite of the many trials and investigations on the prevention of incidence, prevalence and treatment of mastitis, it still remains one of the main challenges (from both the health and economical points of view) in lactating dairy cows. There are a number of reasons including, decreased milk production, medical and management expenses, culling animals, wasted milk from animals treated by antibiotic drugs, and reduction quality of milk [1-3]. High somatic cell count (SCC) is an international standard indicator for sub-clinical mastitis, so individual monitoring of SCC could separate infected cows for treatment or culling [4-6]. While antibiotics have performed a key role in mastitis control on dairy animals, micro-organisms which are emergence resistant to antibiotics have limited their efficacy in management and treatment, thus the use of antibiotics to mask the managerial problems associated with mastitis control should be avoided [7-9]. Other disadvantages include the cultural sensitivity tests used to select the best antibiotic for infection agents with high susceptibility, which are expensive and time consuming, and, further, the residue poses great risk to animal production, adversely affects human health and increases the costs of antibiotic treatment as well [9-13]. On the other hand, antibiotics are recommended only for clinical mastitis, not for the sub-clinical form [14-15]. Further, farmers and consumers have a growing interest in organic dairy farms [16-17]. So economical and residual-free agents like homeopathic medicines are the main alternative to antibiotic therapy which have expanded worldwide. Some of the obtained data has reported that homeopathic treatment could reduce the SCC of lactating cows and provide satisfying results from treating sub-clinical mastitis [10,18-19].

In this study the efficacy of a homeopathic remedy consisting of 2 nosodes (strep. dysgalactiae and staph. aureus) was evaluated in the treatment of sub-clinical mastitis.

MATERIALS AND METHODS

2.1. Farms and Animals

In the first step, a total 600 lactating Holstein cows of a private dairy farm (4400 cows and 2463 lactating cows) around Tehran, Iran were selected based on CMT > +1 and 400,000 ≤SCC ≤ 1000,000 for this study. All of the selected population were in the 1st to 5th lactation period with normally appearing milk and udder, were not suffering from any clinical illness, and no antibiotics or other therapy were used before and during our investigation. This study was planned during spring 2010. In the second schedule (20 days after), CMT was again repeated on the udder quarter of each of the 600 cows, and then between cows which did not decrease SCC and have not shown clinical mastitis; 300 cows were selected. The mean age and weight of the selected cows were 5.5 (3 to 8) years old and 635 (420 to 850) Kg respectively. Udders were washed and dried with water and a cloth, then milked by milking machines twice daily. Cows were randomly classified into two groups, group A (160 cows) with a homeopathic drug containing a combined nosode (strep. dysgalactiae and staph. aureus), administered orally 5ml once daily during a period of 5 days. group B (140 cows), placebo, were given 5ml water at the same time. Before treatment (day zero) and after treatment (day 21, day 28) quarter milk samples were taken for cell count (SCC) and bacteriological culture.

2.2. SCC and bacteriological evaluation

All milk samples were analyzed as follows: SCC evaluation and diagnosis methods were determined by an electronic Fossomatic counter (Model 5000, Foss Fact., Denmark). CMTscore was defined as negative (+0), 1+ (traces), 2+(gel) and 3+(clumps). At the veterinary laboratory, bacteriological examination was conducted according to standard methods [20]. Milk samples were cultured on coated agar plate, mixed with 5% washed bovine erythrocytes (Blood Agar Base, Oxoid Ltd, Hampshive, UK) and incubated aerobically at 37˚c for 24h. Colonies were evaluated by Gram stain, morphology, hemolysis and the number of each colony type. For pure culture, these colonies were sub cultured again. To distinguish Gram-positive cocci, catalase and coagulase production were used, and finally, diagnostics were conducted according to the standard methods of the National Mastitis Council (1999) [21].

2.3. Statistical Analysis

All analyses were performed by SPSS 11.5 (SPSS Inc., Chicago, IL). Data were presented as mean (SD or SE) for quantitative variables and as frequency (percent) for qualitative variables. For bacterial culture, the percents of the infections have been compared in the treatment and control groups by using chi-square test separately on days 0, 21 and 28, and when the assumption of the test did not meet, the exact p-values were computed. Also, for comparing the percents in the time point of day 0, 21 and 28, the Chocran Q test was used in both the treatment and the control groups. For SCC, the normality of the data were evaluated and confirmed by Kolmogorov-Smirnov one sample test. Due to the normality of the data, parametric tests were used afterwards. In the first step, the mean value of the SCCs was compared in the baseline measurements in the two groups and for various lactations to check the homogeneity of the subjects. Two-way analyses of variances (ANOVA) with repeated measurements were performed to investigate the interaction and main effects of the intervention groups and time measures in each lactation. Follow-up tests were done by performing repeated measure ANOVA and independent samples t-tests for testing the time trend and comparing the treatment and control groups respectively. In addition, a series of one–way ANOVA followed by Duncan Post–hoc tests were performed to assess the differences among lactations in the treatment and control groups and in the three time points. P values <0.05 were considered to be significant [22].

RESULTS

3.1. Bacterial culture

The results of the evaluation of the bacterial culture showed significant differences between the treatment and control groups for day 21 and 28 in staph. aureus, strep. dysgalactiae and both (total) of these (All $P<0.05$). However, the results were non-significant for 0 day for these bacteria and the differences were all non-significant for days 0, 21 and 28 in staph. aureus+strep. dysgalactiae (All $P>0.05$) (Table 1) as well. In addition, based on the odds ratios, it can be said that the odds (sub-clinical incidence) of being infected in the treatment group was 67%, 73%, 64%, 74%, 69% and 77% less than that of in the control group for staph aureus 21, staph. aureus 28, strep. dysgalactiae 21, , strep. dysgalactiae 28, total 21 and total 28, respectively (Table 1).

In addition, the results for evaluation of the trends in the 0, 21 and 28 day time points showed significant changes for staph. aureus and the total of staph. aureus and strep. dysgalactiae in the treatment group (both $P<0.05$) (Fig.1 – Fig.4).

Table 1: Summary statistics and the results of comparisons of the percent of infection by various bacteria

Bacteria	Time	Treatment		Control		P-Value	OR (CI)
		Frequency	%	Frequency	%		
st. aureus	0	15	9.40%	13	9.20%	0.963	1.02 (.47 - 2.22)
	21	7	4.40%	17	12.10%	**0.014**	.33 (.13 - .83)
	28	6	3.80%	18	12.80%	**0.004**	.27 (.10 - .69)
st. dysgalactiae	0	14	8.80%	15	10.60%	0.58	.81 (.37 - 1.73)
	21	9	5.60%	20	14.20%	**0.012**	.36 (.16 - .82)
	28	7	4.40%	21	14.90%	**0.002**	0.26(.11 - .64)
st. aureus+st. dys.	0	2	1.30%	2	1.40%	0.899	.88 (.12 - 6.32)
	21	2	1.30%	2	1.40%	0.899	.88 (.12 - 6.32)
	28	1	0.60%	3	2.10%	0.344	.29 (.03 - 2.81)
Total	0	29	18.10%	28	19.90%	0.702	.89 (.50 - 1.59)
	21	16	10.00%	37	26.20%	**<.001**	.31 (.17 -.59)
	28	13	8.10%	39	27.70%	**<.001**	.23 (.12 - .46)

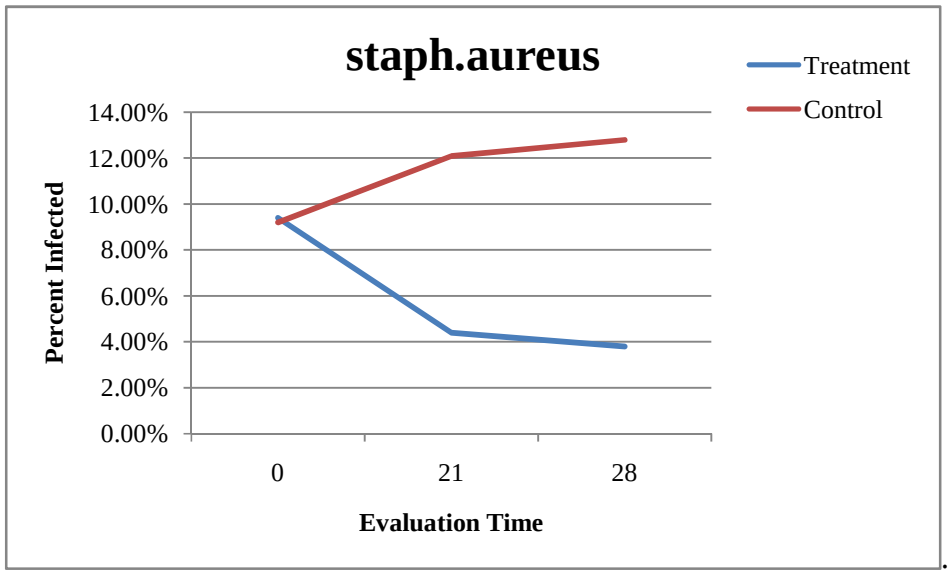

Fig 1. Time trend of Percent of infected in bacteria culture for st.aureus

3.2. Somatic cell count (SCC)

The results of the K-S test for normality showed that this variable was normal for all periods of lactation and for the treatment and control groups (All P>0.05). In the first step, the baseline measurements were compared to investigate the homogeneity of the subjects in the treatment and control groups in this measurement. Results of the test showed no significant differences between these groups (All P>0.05) separately in each period of lactation, also there were no significant differences among the five periods of lactation in both the treatment and control (Both P>0.05) groups and hence the homogeneity of the subjects is confirmed (Table 2).

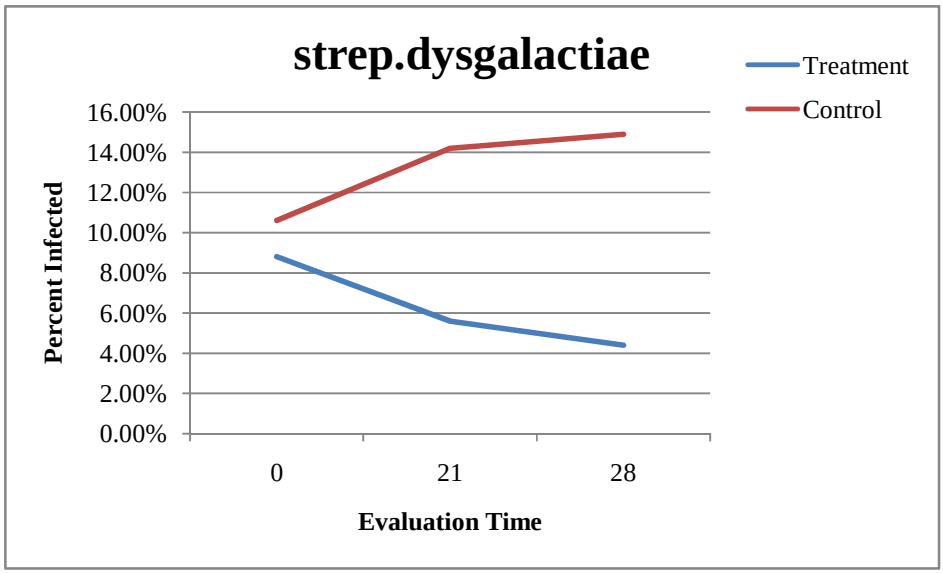

Fig 2. Time trend of Percent of infected in bacteria culture for st.dysgalactiae

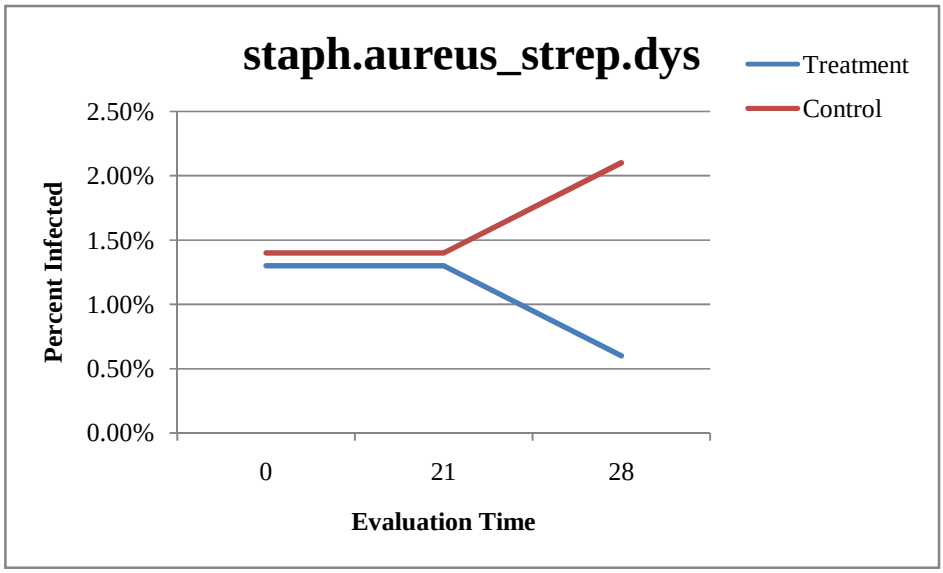

Fig 3. Time trend of Percent of infected in bacteria culture for st.aureus_st.dys

There were Measurements in time by group interactions for all periods of lactation (All P<0.05), (Table 3), hence for all periods of lactation the time trends differ in the treatment and control groups. Therefore, a series of analyses were performed to compare the mean value of SCC between the treatment and control groups, and to compare the mean value of SCC in three time windows separately in these groups for each period of lactation. In addition, a series of analyses were performed to compare the mean value of SCC among the periods of lactation, and to compare the mean value of SCC in three time windows separately in each period of lactation or the treatment and control groups.

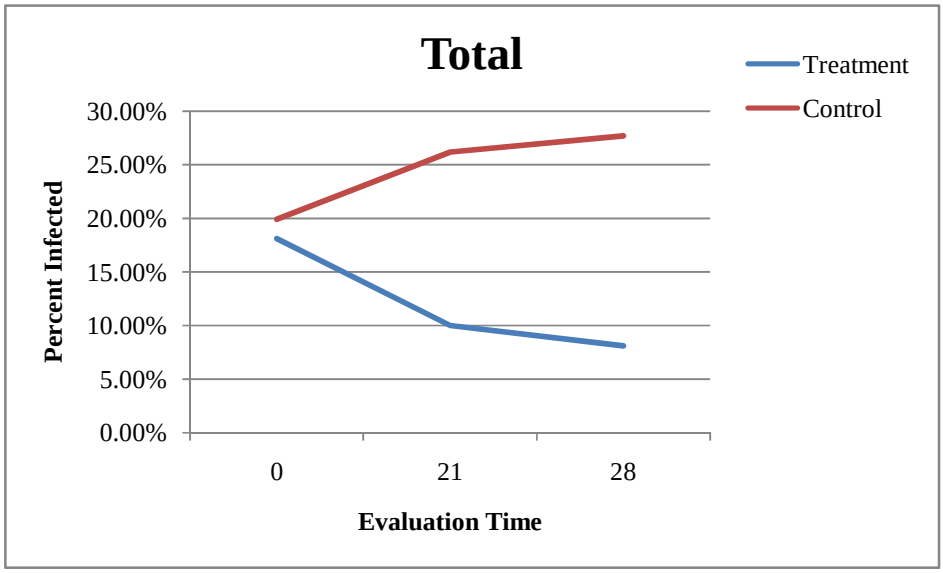

Fig 4. Time trend of Percent of infected in bacteria culture for Total

Table 2: Summary statistics and the results of comparisons of the mean for SCC in baseline measurements

Lactation	Group	Mean	Std. Deviation	P-Value@
1	Treatment	641.84	147.799	.124
	Control	624.59	176.053	
2	Treatment	606.05	147.455	.616
	Control	596.97	162.329	
3	Treatment	630.20	158.905	.420
	Control	690.37	169.645	
4	Treatment	665.38	163.052	.993
	Control	625.10	159.166	
5	Treatment	708.30	187.448	.152
	Control	602.50	157.874	
Results for comparing lactation	Treatment	$F_{(4, 155)}$ = 1.66 ,P-Value =.163		
	Control	$F_{(4, 136)}$ = 1.46 ,P-Value =.217		

@ : P-Value based on independent samples T-test for comparing treatment and control groups

3.2.1. Results for comparing treatment and control groups in each time separately for each period of lactation

Significant differences were observed between the treatment and control groups for all periods of lactation on 21 and 28 days (All P>0.05), but the differences were not significant between these two groups in time 0 (Table 3).

3.2.2. Results for comparing mean of time points in treatment and control groups separately for each period of lactation

For first lactation**,** there were significant changes for mean of SCC in times 0, 21 and 28 days in both the treatment and control groups (both P<0.05, Table 3) and the results of the Sidak test showed that in both the treatment and control groups, all three time points were significant when compared with another (All P<0.05). For lactation 2**,** there were significant changes in the mean of SCC in times 0, 21 and 28 days only in the treatment group (P<0.05, Table 3), and the results

of the Sidak test showed that, all three time points showed significant changes when compared with one another (All P<0.05). For lactation 4**,** the changes in the mean of SCC were significant in times 0, 21 and 28 days in both the treatment and control groups (both P<0.05, Table 3) and the results of the Sidak test showed that in both the treatment and control group, all three time points showed marked changes, with one another (All P<0.05). For lactation 5**,** there were significant changes in the mean of SCC in times 0, 21 and 28 days only in the treatment group (P<0.05, Table 3), and the results of the Sidak test showed that, all three time points were considerable, with one another (All P<0.05). However, for the control group in lactation 2**,** the treatment and control groups in lactation 3, and the control group in lactation 5, no significant changes in time were observed (All P>0.05, Table 3).

3.2.3. Results for comparing periods of lactation in time windows and separately for treatment and control groups

In all three time points, there were significant differences among the five periods of lactation in the treatment group (All P<0.05, Table 3). In addition, the results of Duncan post hoc tests showed that for SCC in time 0, there were significant differences between lactation 1 and lactations 4 and 5, and between lactation 2 and lactation 5 (All P<0.05). For SCC in time 21, there was a significant difference between lactation 1 and lactation 4 (P<0.05), and for SCC in time 28, there significant differences between lactation 1 and lactation 4, as well as significant differences between lactation 3 and lactation 4 (All P<0.05).

Table 3: Summary statistics and the results of the analyses for SCC in 0, 21 and 28 days

Lactation	Time	Group	N	Mean	Std. Deviation	P-Value@	Results for testing Group-Birth Interaction
1	0	Treatment	19	585.63	133.36	.064	$F_{(2, 82)}$ = 134.55 , **P-Value <0.001**
		Control	29	673.28	186.10		
	21	Treatment	19	338.21	84.88	**<.001**	
		Control	27	691.74	230.42		
	28	Treatment	19	305.21	83.62	**<.001**	
		Control	25	696.84	259.92		
	Results for testing Time trend	Treatment	$F_{(2, 34)}$ = 4.01,**P-Value =.027**				
		Control	$F_{(2, 46)}$ = 10.28 ,**P-Value <.001**				
2	0	Treatment	41	625.27	146.32	.654	$F_{(2, 126)}$ = 170.56, **P-Value <0.001**
		Control	30	641.87	163.00		
	21	Treatment	41	396.34	128.23	**<.001**	
		Control	25	693.68	192.28		
	28	Treatment	41	373.24	159.71	**<.001**	
		Control	25	727.04	208.58		
	Results for testing Time trend	Treatment	$F_{(2, 78)}$ = 3.59,**P-Value =.032**				
		Control	$F_{(2, 46)}$ =.11 ,P-Value =.899				
3	0	Treatment	30	663.40	185.35	.291	$F_{(2, 104)}$ = 134.55 , **P-Value <0.001**
		Control	30	712.93	174.26		
	21	Treatment	29	383.07	97.41	**<.001**	
		Control	27	744.96	199.78		
	28	Treatment	29	342.97	99.96	**<.001**	

		Control	26	777.69	226.24		
	Results for testing Time trend	Treatment	$F_{(2, 54)}$ = 2.24 ,P-Value =.116				
		Control	$F_{(2, 48)}$ =.02 ,P-Value =.982				
4	0	Treatment	50	714.10	188.68	.321	$F_{(2, 82)}$ = 221.39, **P-Value <0.001**
		Control	30	671.80	174.01		
	21	Treatment	48	489.50	247.46	**<.001**	
		Control	29	702.55	226.29		
	28	Treatment	47	464.89	273.15	**<.001**	
		Control	29	732.10	243.49		
	Results for testing Time trend	Treatment	$F_{(2, 90)}$ = 5.10,**P-Value =.008**				
		Control	$F_{(2, 54)}$ =7.63 ,**P-Value =.001**				
5	0	Treatment	20	765.30	185.60	.056	$F_{(2, 146)}$ = 58.44, **P-Value <0.001**
		Control	22	654.64	178.79		
	21	Treatment	18	477.56	138.88	**.001**	
		Control	19	691.79	211.29		
	28	Treatment	18	438.78	128.75	**.001**	
		Control	16	670.31	202.31		
	Results for testing Time trend	Treatment	$F_{(2, 32)}$ = 971,**P-Value =.001**				
		Control	$F_{(2, 28)}$ =.09 ,P-Value =.914				
Results for comparing lactations	0	Treatment	**$F_{(4, 155)}$ = 4.21,P-Value =.003**				
		Control	$F_{(4, 136)}$ = 0.69,P-Value =.603				
	21	Treatment	**F(4, 150) = 4.20,P-Value =.003**				
		Control	$F_{(4, 122)}$ = 0.30,P-Value =.878				
	28	Treatment	**F(4, 149) = 3.73,P-Value =.006**				
		Control	$F_{(4, 116)}$ = 0.66,P-Value =.621				

@ : P-Value based on independent samples T-test for comparing treatment and control groups

DISCUSSION

In lactating dairy cows, subclinical mastitis is one of the most frequent and costly illnesses in the dairy industry. With regards to the ethical concerns and financial losses from antibiotic residues in milk, meat and, on the other hand, the published promising results [16-17], this study evaluated the efficacy of homeopathic nosodes formulated for streptococcus dysgalactiae and staphylococcus aureus on subclinical mastitis. After treatment, the SCC was lower in the treated group compared with the control group.

Homeopathic treatment consisting of a herd remedy and two autogenous nosodes of mastitis causing organisms reduced the SCC in lactating dairy cows. An autogenous nosode of mastitis causing organisms seemed to be equally effective as dry-cow antibiotic therapy for the prevention of subclinical mastitis [23]. Dhakal, (2006) has pointed out that SCC is always compared with bacteriology and these tests are almost never complete agreement [24]. Some studies have demonstrated that staphylococcus aureus is associated with more greatly elevated SCC than other staphylococci, and microbial cultures of individual or mixed quarter milk samples are used in diagnosing mastitis in bovine[6,25] and the control of mastitis caused by strep. Aglactiae and staph.aureus have resulted in reductions in bulk tank somatic cell count [26]. The most isolated bacteria in dairy cow mastitis were staphylococcus, streptococcus SPP. and C.bovis [27-29]. Some studies showed staphylococcus aureus was the most important

microorganism responsible for mastitis [30-31]. In a subclinical mastitis survey, after staphylococcus aureus, the second most frequently isolated bacterium was streptococcus dysgalactiae [32], which are significant pathogens related to bovine mastitis in lactating and nonlactating dairy cows, and are widely contagious in dairy herds [33]. In early lactation, these infected cows had a higher SCC, reduced milk production and culling risk compared with the culture negative cows of calving [34-35]. Whist et al 2007 have reported streptococcus dysgalactiae-positive cows had a significantly higher SCC and approximately 334 Kg less milk over a 305-day lactation compared with culture-negative cows [36]. Controlling these pathogens by treatment strategy during lactation may be one solution [37-38]. This study revealed a remarkably high and endemic presence of streptococcus dysgalactiae and staphylococcus aureus in the cattle population. The results of the evaluation of the bacteria culture in this study showed significant differences between the treatment and control group for day 21 and day 28 in staph.aureus, strep.dysgalactiae and the total of both of these. The clinical efficacy of homeopathic nosodes in the treatment of subclinical mastitis is in agreement with the earlier observation and could be ascribed to the Fernando Moncayo et al (2001) studies which showed that homeopathic treatment consisting of a herd remedy and autogenous nosodes (streptococcus dysgalactiae and staphylococcus aureus) of mastitis causing organisms significantly ($p<0.05$) reduced the SCC in lactating dairy cows [23]. In contrast, Klocke et al (2000) claimed there are no beneficial efforts after combined homeopathic therapy with tuberculium nosode on subclinical mastitis [18]. Egan et al 1998 evaluated the efficacy of nosodes formulated for strep. agalactiae, strep. dysgalactiae strep.uberis, staph. aureus, and Escherichia coli and concluded that the nosodes had no effect in reducing the mastitis incidence or milk SCC [5]. Day claimed to find some benefits from the use of a nosode for an unspecified period in three herds, while Sonnewald found homeopathic preparations were more successful than antibiotics in treating mastitis cases caused by Gram-negative bacteria, but less effective than antibiotics in treating mastitis cases caused by Gram-positive bacteria[39-40]. In a field study of 100 cases of acute clinical mastitis 2 it was found that homeopathic treatment gave similar cure rates to antibiotics [41]. In this study, 1.30 % of the cows had mixed infection with strep. dysgalactiae and staph. aureus within the same quarter. One study claimed there was no significant association between the mixed infection and CMT, SCC and milk yield [23]. In cases of non-fibrosed clinical mastitis, the average quarter cure rate of animals treated with antibiotics was lower (59.2%) than that (86.6%) of those treated with the homeopathic combination medicine, but the mean recovery period in cows treated with homeopathic combination medicine was significantly longer than the average recovery period of cows treated with antibiotics [3]. The question of whether the cost could be reduced by treating subclinical mastitis in these cows could be answered in this study based on SCC and the bacteriological results. Homeopathic medicine could be an economical and acceptable method to avoid additional costs. The average total cost of therapy was significantly lower with the homeopathic combination medicine than with antibiotics [3].

Besides the potential for self cure and treatment with homeopathy remedies, other physical methods like cooling, milking out, massage, and/or ointments could be effective in achieving satisfying results [18-19]. Further analysis and comparison of other differences in nosodes both with and without physical treatment is necessary in order to provide better results [18-19]. On the other hand, it is recommended that risk factors like age, days postpartum, and season as important factors of subclinical mastitis, be considered with nosodes homeopathic treatment.

CONCLUSION

It was concluded that treatment of cows with subclinical mastitis using a combination of homeopathic nosodes resulted in lower SCC and reduced isolated bacteria compared with the control group. In addition, the nosodes had an effect in reducing the incidence of mastitis.

Acknowledgment
The authors would like to thank the participating farmers, veterinarians, and laboratory workers for their assistance and support during the trial.

REFERENCES

[1] R. Searcy, O. Reyes, G.Guajardo, *British Homoeopathic Journal*, **1995**, 84, 67-70.
[2] M. Silhava, M. Soch, D. Lukesova, *Agriculturea tropica Et Subtropica*, **2005**, 38, 57-63.
[3] J.P. Varshney, R. Naresh, *Homeopathy*, **2005**, 94, 81-85.
[4] M. Koivula, E.A. Mäntsysaari, E. Negussie, T. Serenius, *J. Dairy Sci.*, **2005**, 88, 827-833.
[5] J.S. Moon, H.C. Koo, Y.S. Joo, S.H. Jeon, D.S. Hur, C.I. Chung, H.S. Jo, Y.H.Park, *J. Dairy Sci.*, **2007**, 90, 2253-2259.
[6] B. Djabri, N. Bareille, F. Beaudeau, H. Seegers, *Vet. Res*., **2002**, 33, 335-357.
[7] J. Egan. *Irish veterinary journal*, **1998**, 51, 141-143.
[8] M. Sandholm, L. Kaartinen, M.Pyorala, *Journal of Veterinary Pharmacology and Theraputhics*, **1990**, 13, 248-260.
[9] M. Hovi, *Vet. Rev*. **2000**, 56, 226-229.
[10] A. Busato, P.Trachsel, M.Schällibaum, J.W. Blum, *Preventive Veterinary Medicine*, **2000**, 44, 205-220.; W. Owens, J. Watts, R. Boddie, S. Nickerson, *J. Dairy Sci*., **1988**, 71, 3143-3147.
[11] M. Hurst, *Cattle Pract*., **2000**, 8, 279-282.
[12] A.M. Biggs, *Cattle Pract*., **2000**, 8, 283-285.
[13] L.Stdnik, F. Louda, R. Tousova, F. Viedemann, A. Slegr, *Farmer*, **2000**, 12, 35-36.
[14] W. Owens, J. Watts, R. Boddie, S. Nickerson, *J. Dairy Sci*., **1988**,71,3143-3147.
[15] A.O. Refsdal, *Animal Rep. Sci*., **2000**, 60-61, 109-119.
[16] M.J.Haskell, F.M. Langford, M.C.Jack, L. Sherwood, A.B. Lawrence, K.M.D. Ruth, *J. Dairy Sci.*, **2009**, 92, 3775-3780.
[17] J.P. Varshney, R. Naresh, *Homeopathy*, **2004**, 93, 17-20.
[18] P. Klocke, S. Garbe, J. Spranger, C.C. Merck. in: 13th International IFOM Scientific Conference, Basel, **2000**, 2000b, 343.
[19] P. Klocke, S. Ivemeyer, F. Heil, M. Walkenhorst, C. Notz, in: 3rd QLIF Congress, Hohenheim, Germany, **2007**.
[20] G.A. Houghtby, L.J. Maturin, E.K. Koenig, 16th ed. R.T. Marshall, ed. Am. Publ. Health Assoc., Inc., Washington, DC. PP 213-246.
[21] National Mastitis Council, Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI, **1999**.
[22] J.H. Zar. Biostatistical Analysis, Fifth Edition **1998**; New York: Pearson Press.
[23] F. Moncayo, *OFRF Information Bulltin*, **2001**, 10, 24-28.
[24] I.P. Dhakal, *J. Vet. Med*., **2006**, Series B, 53, 2, 81-86.
[25] P. Moroni, G. Pisoni, G. Ruffo, P.J. Boettcher, *Prev. Vet. Med*., **2005**, 69, 163-173.

[26] D.L. Nash, G.W. Rogers, J.B. Cooper, G.L. Hargrovc, J.F. Keown, *J.Dairy Sci*., **2002**, 85, 1273-1284.
[27] D.J.Wilson, R.N. Gonzalez, H.H. Das, *J.Dairy Sci.*, **1997**, 80, 2592-2598.
[28] P.J. Mylrea, R.J.T. Hoare, P. Colquhoun, I.J. Links, R.J. Richards, M. Barton, *Aust. Vet. J.*, **1977**, 53, 534.
[29 C.D. Wilson, M.S. Richards, *Vet. Rec*., **1980**, 106, 431.
[30] M.S. Jaffery, A.R. Rizvi, *Acta Trop*, **1975**, 32, 75-78.
[31] H. Unnerstad, A.Lindberg, K.P. Waller, T. Ekman, K.Artursson, M. Nilsson-ost, B. Bengtsson, *Vet. Micro*., **2009**, 137, 90-97.
[32] O. Østerås, l. Sølverød, O. Reksen, *J.Dairy Sci*., **2006**, 89, 1010-1023.
[33] International Dairy Federation. Suggested interpretation of mastitis terminology- Bull., 338, Belgium: IDF, Brussels, **1999**.
[34] R.J. Harmon, *J. Dairy Sci*., **1994**, 77, 2103–2112.
[35] Y. De Haas, H.W. Barkema, R.F. Veerkamp, *J. Dairy Sci*., **2002**, 85, 1314–1323.
[36] A.C. Whist, o. Østerås, l. Sølverød, *J.Dairy Sci*., **2007**, 90, 766-778.
[37] S.P, Oliver, B.E. Gillespie, S.J. Headrick , H. Moorehead , P. Lunn , H.H Dowlen , *J. Dairy Sci.*, **2004**, 87, 2393–2400.
[38] S.G. St. Rose, J.M Swinkels , W.D. Kremer, C.L. Kruitwagen, R.N. Zadoks, *J. Dairy Res*., **2003**,70, 387–394.
[39] C.E.I. Day, *Journal for Veterinary Homeopathy*, **1986**, 1, 15-19.
[40] B.M. Sonnenwald, Abstract 1438 *Dairy Sci. Abstracts*, **1986**, 50, 158.
[41] J.P.Varshney, R.Naresh, *Homeopathy*, **2005**, 94, 81-85.