Iranian Journal of Veterinary Research, Shiraz University, Vol. 12, No. 3, Ser. No. 36, 2011

Short Paper

The prevalence of clinical and subclinical mastitis in dairy cows in the central region of Fars province, south of Iran

Hashemi, M.[1*]; Kafi, M.[2] and Safdarian, M.[1]

[1]Department of Animal Sciences, Research Center for Agriculture and Natural Resource of Fars Province, Shiraz, Iran; [2]Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]**Correspondence:** M. Hashemi, Department of Animal Sciences, Research Center for Agriculture and Natural Resource of Fars Province, Shiraz, Iran. E-mail: Hashemi@farsagres.ir

(Received 18 Apr 2010; revised version 19 Jan 2011; accepted 26 Jan 2011)

Summary

Mastitis continues to be one of the economically most important diseases in dairy farming. Forty-six licensed dairy farms in the central region of Fars province were randomly selected in order to participate in a seasonal prevalence study. A cross-sectional study was designed to determine prevalence at cow and quarter level based on clinical signs for clinical mastitis and indirect tests for subclinical mastitis. 6180 quarters from 1545 dairy cows were tested by clinical examination and California mastitis test (CMT). Milk samples from both clinical and subclinical quarters were collected for bacteriological culture. 4714 (76.28%) quarters were healthy, 1335 (21.6%) quarters were positive by results of CMT (as indicated to subclinical mastitis), 44 (0.71%) quarters showed clinical mastitis signs and 87 (1.41%) quarters were blind. The clinical and subclinical mastitis prevalence at cow level was 2.2 and 42.5%, respectively. There was no significant difference in the prevalence of mastitis between different quarters, seasons and cities. The most prevalent isolated bacteria were coagulase positive staphylococci followed by Streptococci, *Escherichia coli* and coagulase negative staphylococci. Insufficient control measures such as pre and post milking hygiene and dry cow therapy in dairy farms and limited knowledge of farmers on the importance, identification and control of mastitis, especially subclinical forms, can be the main causes for the high prevalence of mastitis in Fars province.

Key words: Mastitis, Prevalence, Dairy cows, Fars, Iran

Introduction

Mastitis is defined as an inflammation of the parenchyma of mammary gland, which can reduce milk yield and alter milk composition (Souto *et al*., 2010). There are two main classes of mastitis. The first is clinical mastitis, which manifests signs observed from the animal or the milk. The other is subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools.

Since the quality and quantity of the milk is influenced by mastitis, it is considered to be one of the most important causes of economic losses in the dairy industry worldwide. Mammary gland infections cost the US dairy industry approximately 2 billion dollars annually and have a similar impact in Europe (Donovan *et al*., 2005).

Somatic cells are part of the natural defense mechanism and include lymphocytes, macrophages, polymorpho-nuclear cells and some epithelial cells (Pillai *et al*., 2001). Somatic cell count (SCC) can be measured quantitatively by California mastitis test (CMT). It is a simple, easy and low cost screening test for subclinical mastitis at dairy farms (Dingwell *et al*., 2003). Validity of CMT in diagnosis of infected quarters was established in various milking stages (Dingwell *et al*., 2003; Gharagozloo *et al*., 2003).

Mastitis control program can reduce economical loss and increase herd efficacy and milk hygiene. Epidemiological data including mastitis prevalence, mastitis-causing organisms, predisposing factors and response to treatment, are necessary for the establishment of a mastitis control program, but no published research is available about mastitis in Fars province (in the south of Iran). This province has captured the first rank for milk production (424 million liters annually) in the south of Iran (Agriculture Statistics of Iran, 2007). In spite of the considerable production of milk in this region, most cows are being kept on small farms with undesirable housing and milking parlor sanitation. This study was carried out to determine mastitis prevalence and mastitis-causing organisms in dairy farms of Fars province.

Materials and Methods

Study area

This study was carried out in the central region of Fars province (in the south of Iran) where 64.57% of Fars' raw milk is produced. All of the licensed dairy farms (46) were visited in Shiraz (17), Marvdasht (19) and Sepidan (10) cities and 1545 milking cows were examined (535, 620 and 390 heads in Shiraz, Marvdasht and Sepidan, respectively). All cows were milked by machine. A quartile of dairy farms in each city was randomly selected during a period from March 2003 to February 2004 in order to participate in the seasonal prevalence study.

Prevalence study

A cross-sectional study was designed to determine prevalence at cow and quarter level based on clinical signs for clinical mastitis and indirect tests for subclinical mastitis. Selected dairy farms were visited during morning milking and individual quarter milk samples from all lactating cows above 30 days postpartum were examined in the milking parlour. Thereafter, when the cows entered the milk parlour, their quarters had been washed with tap water, dried by towel separately and their first three milking streams discarded. Clinical mastitis was detected by gross signs including warm, hard and swollen quarter and abnormal appearance of milk. CMT was used to detect subclinical mastitis. Two milliliters of milk from each udder quarter was milked in a plate that had four separated cups. Three milliliters CMT liquid (Delaval, Poland) was added to each cup and mixed gently by rotating the plate. The reaction was then visually scored depending upon the amount of gel formation as follows:

Negative = no reaction
Trace = appearance of streaks can be made visible during rotation of the plate
1+ = distinct thickening during rotation, but no gel
2+ = slight formation of gel which follows the rotating plate very slowly
3+ = solid formation of gel that adheres to the base of the plate

Quarters that scored negative and trace were assumed healthy and the quarters with different positive scores were assumed infected. A cow with at least one affected quarter at the time of examination was considered to have either clinical or subclinical mastitis. When a farm had a cow with mastitis, it was accounted to have mastitis. Only one of the infected quarters from each cow was selected for milk sampling except when the cow had four severely infected quarters so one more milk sample was collected. Then the teat end of the selected quarter was swabbed with cotton soaked in 70% ethyl alcohol and 10 milliliters of milk was approximately collected into sterile containers. Samples were transported to the laboratory in a special box with ice at 4°C for bacteriological investigation (Quinn *et al*., 1994).

Bacteriology

Bacterial culture was performed according to Quinn *et al*. (1994). Milk samples from both clinical and subclinical cases were streaked on blood and MacConkey agar plates under 24 h after sampling, then incubated under aerobic conditions at 37°C and analysed at 24 and 48 h.

Bacteria on culture-positive plates were identified according to their size, shape, color, hemolytic characteristics, Gram's reaction and catalase production of colonies. For confirmation, different biochemical tests were used following subculturing and isolation of distinct colonies. The culture was considered negative if no growth occurred after 48 h of incubation. Isolation of two or more types of colonies from a quarter was considered mixed growth and the result was disregarded.

Data analysis

All statistical analysis was carried out in SPSS for windows 11.5.0 (SPSS Inc., 2002).

Data were analysed descriptively in the first step. Then the association of the different variables with the prevalence of mastitis at the cow or quarter levels was analyzed using a Chi-squared test. The level of significance was set at $P<0.05$.

Results

Prevalence

Clinical examination and CMT was carried out on 6180 quarters from 1545 cows. Frequency distribution of healthy, blind and infected quarters is shown in Table 1. Among 1545 examined lactating cows, clinical and subclinical mastitis prevalence at cow levels was 2.2 and 42.5%, respectively. There was no significant difference between seasons and cities ($P>0.05$). Clinical and subclinical mastitis prevalence at quarter level did not significantly ($P>0.05$) differ between seasons, cities and quarter location (Table 2). Subclinical and clinical mastitis was detected in 100 and 41.6% of farms, respectively.

Bacteriology

Milk samples from 32 (72.7%) quarters with clinical mastitis signs and 694 (52%) quarters with various degree of positive CMT were cultured. 535 (73.69%) samples were culture positive, 12 (1.65%) were mixed growth and 179 (24.66%) showed no bacterial growth. The results of the bacteriological findings of milk samples of infected quarters are described in Table 3. The most prevalent isolated bacteria were coagulase positive staphylococci followed by *Escherichia coli*, Streptococci and coagulase negative staphylococci.

Discussion

In the present study, like some other studies, the majority of the cases of mastitis were subclinical (Almaw *et al*., 2008; Getahun *et al*., 2008). It may be due to a higher knowledge of farmers on clinical mastitis which appears by visible changes and is treated as soon as possible. The prevalence of clinical mastitis at cow level in this study (2.2%) was comparable with

Table 1: Frequency distribution of healthy, blind and infected quarters by mastitis in examined dairy cows in the central region of Fars province

Quarter	Healthy		Subclinical		Clinical		Blind		Total
	No.	%	No.	%	No.	%	No.	%	No.
Right-front	1220	19.74	297	4.81	8	0.13	20	0.32	1545
Right-back	1205	19.5	303	4.9	10	0.16	27	0.44	1545
Left-front	1139	18.43	373	6.04	14	0.23	19	0.31	1545
Left-back	1150	18.61	362	5.86	12	0.19	21	0.34	1545
Total	4714	76.28	1335	21.6	44	0.71	87	1.41	6180

Table 2: The mean of mastitis prevalence percentage at cow and quarter levels in dairy farms in the central region of Fars province

Mastitis	Season				City			Quarter location			
	Spring	Summer	Autumn	Winter	Shiraz	Marvdasht	Sepidan	Right-front	Right-back	Left-front	Left-back
Cow level											
Subclinical	44.2	45.94	40.14	48.89	45.93	44.07	44.57				
Clinical	1.92	2.41	3.22	1.74	2.78	1.9	2.25				
Quarter level											
Subclinical	21.3	21.86	21.92	25.68	24.5	21.84	21.41	21.13	20.93	25.34	23.5
Clinical	0.52	0.69	1.04	0.57	0.71	0.61	0.85	0.5	0.5	0.89	0.91

Table 3: Bacteriological finding in milk samples from infected quarters by mastitis in dairy farms in the central region of Fars province

Bacteria	Subclinical		Clinical		Total	
	No	%	No	%	No	%
cog+ Staphylococci	136	19.6	6	18.75	142	19.56
Streptococci	94	13.54	5	15.63	99	13.64
Escherichia coli	94	13.54	2	6.25	96	13.22
Cog- Staphylococci	91	13.11	0	0	91	12.53
Bacillus spp.	35	5.04	1	3.13	36	4.96
Corynebacterium spp.	22	3.17	0	0	22	3.03
Yersinia spp.	11	1.59	2	6.25	13	1.79
Proteus spp.	9	1.3	1	3.13	10	1.38
Klebsiella spp.	7	1.01	1	3.13	8	1.10
Pseudomonas spp.	5	0.72	0	0	5	0.69
Acinetobacter spp.	5	0.72	0	0	5	0.69
Micrococcus spp.	4	0.58	1	3.13	5	0.69
Pasteurella spp.	2	0.29	0	0	2	0.28
Enterococcous spp.	1	0.14	0	0	1	0.14
Mixed growth	6	0.86	6	18.75	12	1.65
Negative	172	24.78	7	21.88	179	24.66
Total	694	100	32	100	726	100

that of Kivaria *et al*. (2004) and Almaw *et al*. (2008), who reported 3.8 and 3.9% in Tanzania and Ethiopia, respectively. However, the present finding was lower than that reported by Dego and Tareke (2003) (37.1%).

Clinical mastitis was diagnosed in an average of 9.9%, range 0.9%-21.4% of calved cows within the Estonian dairy herd. The prevalence of clinical mastitis at quarter level in this study (0.71%) is in agreement with the report of Getahun *et al*. (2008) (0.51%), but it is lower than the findings of Dego and Tareke (2003) (39.2%). These differences may be explained by different management factors such as specific dry period management strategies (Green *et al*., 2007), leaking milk and previous udder infection (Mungube *et al*., 2005), and feeding regimes and heifer replacement rates (McDougall, 1999).

The results showed a high prevalence of subclinical mastitis (42.5%) in dairy farms of Fars province that was higher than earlier reports from Semnan (33.2%) and Isfahan (33.7%) provinces in Iran (Rafyi-Barzoki, 1998; Zamani *et al*., 2004). Poor management in a high percentage of small dairy farms in Fars province may be the main cause for this difference. The prevalence of subclinical mastitis in Uruguay was reported 52.4% as measured on a cow basis and 26.7% as measured on a quarter basis, which is higher than our results (Gianneechini *et al*., 2002). Two reports from different regions of Ethiopia showed prevalence of subclinical mastitis 22.3 and 34.4% at cow level and 10.1 and 4.9% at quarter level (Almaw *et al*., 2008; Getahun *et al*., 2008) that were lower than our results in Fars province, but Dego and Tareke (2003) reported 62.9% (on the basis of cow) and 60.8% (on the basis of quarter) prevalence in southern Ethiopia. In other parts of Africa, Kivaria *et al*. (2004) and Karimuribo *et al*. (2008) reported a higher prevalence of subclinical mastitis in lactating cows in small holder farms of Tanzania (90.3 and 75.9% at cow and 84.5 and 46.2% at quarter level, respectively). The percentage of cows with subclinical mastitis measured by SCC was 52.2% in Estonia dairy farms (Haltia *et al*., 2006). Subclinical mastitis is a complex disease and the differences in results could be due to differences in management systems between farms, stage of lactation, parity, breed (Almaw *et al*., 2008), severe teat end lesions (Siber and Farnsworth, 1981) and milking hygiene (Haltia *et al*., 2006).

Staphylococci were the predominant bacteria as the cause of mastitis in Fars province, which is in agreement with the findings of Meranzadeh *et al*. (2001) and

Gharagozloo *et al*. (2003) in other regions of Iran. Isolation rate of *Staphylococcus aureus* was also the highest in the reports from different regions of Ethiopia (Getahun *et al*., 2008), Uruguay (Gianneechini *et al*., 2002) and Canada (Olde Riekerink *et al*., 2008). In our study, coagulase negative staphylococci was the fourth most common isolated bacteria, which is in contrast to the findings of Atyabi *et al*. (2006) and Almaw *et al*. (2008) who introduced coagulase negative staphylococci as the most common isolated bacteria. Isolation of both environmental and contagious pathogens address insufficient control measures such as pre and post milking hygiene and dry cow therapy in dairy farms in Fars province and limited knowledge of farmers on the importance, identification and control of mastitis, especially the subclinical form. It emphasizes the need to design extension programs with the aim of increasing farmers' awareness about mastitis.

In light of our findings it can be concluded that the prevalence of mastitis is high and both contagious and environmental pathogens are involved in Fars province. Regularly monitoring of udder health status, housing and milking parlor sanitation, appropriate pre and post milking practice, and dry cow therapy and culling are essential as well to achieve an efficient control program. Awareness should also be increased among dairy farmers regarding the economic impacts and benefits of controlling mastitis.

Acknowledgement

The authors would like to thank Mr. M. R. Sarvghad for his technical assistance in the bacteriological diagnosis.

References

Agriculture Statistics of Iran (2007). *The yearbook of agriculture statistics of Iran. Bureau of statistics and information technology*. 1st Edn., Tehran, Iran, The Ministry of Jihad-E-Agriculture. (In Persian). P: 72.

Almaw, G; Zerihun, A and Asfaw, Y (2008). Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Trop. Anim. Health Prod., 40: 427-432.

Atyabi, N; Vodjgani, M; Gharagozloo, F and Bahonar, A (2006). Prevalence of bacterial mastitis in cattle from the farms around Tehran. Iranian J. Vet. Res., 7: 76-79.

Dego, OK and Tareke, F (2003). Bovine mastitis in selected areas of southern Ethiopia. Trop. Anim. Health Prod., 35: 197-205.

Dingwell, RT; Leslie, KE; Schukken, YH; Sargeant, JM and Timms, LL (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: 413-415.

Donovan, DM; Kerr, DE and Wall, RJ (2005). Engineering disease resistant cattle. Transgenic Res., 14: 563-567.

Getahun, K; Kelay, B; Bekana, M and Lobago, F (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. Trop. Anim. Health Prod., 40: 261-268.

Gharagozloo, F; Blourchi, M; Tabatabaee, AM; Ghasemzadeh Nava, H and Vojgani, M (2003). Sensivity and specifity California mastitis test to detect subclinical mastitis in dairy cows. Pajouhesh and Sazandegi. 59: 59-62 (In Persian with English abst.).

Gianneechini, R; Concha, C; Rivero, R; Delucci, I and Moreno López, J (2002). Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. Acta Vet. Scand., 43: 221-230.

Green, MJ; Bradley, AJ; Medley, GF and Browne, WJ (2007). Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. J. Dairy Sci., 90: 3764-3776.

Haltia, L; Honkanen-Buzalski, T; Spiridonova, I; Olkonen, A and Myllys, V (2006). A study of bovine mastitis, milking procedures and management practices on 25 Estonian dairy herds. Acta Vet. Scand., 48: 22.

Karimuribo, ED; Fitzpatrick, JL; Swai, ES; Bell, C; Bryant, MJ; Ogden, NH; Kambarage, DM and French, NP (2008). Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania. Vet. Rec., 163: 16-21.

Kivaria, FM; Noordhuizen, JP and Kapaga, AM (2004). Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. Trop. Anim. Health Prod., 36: 581-592.

McDougall, S (1999). Prevalence of clinical mastitis in 38 Waikato dairy herds in early lactation. N. Z. Vet. J., 47: 143-149.

Meranzadeh, H; Moslehi, S; Hedari, A; Zahedi, N and Tawassoli, A (2001). Evaluation of the isolated bacteria from raw milk contaminated by mastitis and its importance. *Proceedings of the first specialized dairy industry symposium*. 10-11 November, Tehran, Iran. PP: 83-94 (In Persian with English abst.).

Mungube, EO; Tenhagen, BA; Regassa, F; Kyule, MN; Shiferaw, Y; Kassa, T and Baumann, MP (2005). Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. Trop. Anim. Health Prod., 37: 503-512.

Olde Riekerink, RG; Barkema, HW; Kelton, DF and Scholl, DT (2008). Incidence rate of clinical mastitis on Canadian dairy farms. J. Dairy Sci., 91: 1366-1377.

Pillai, SR; Kunze, E; Sordillo, LM and Jayarao, BM (2001). Application of differential inflammatory cell count as a tool to monitor udder health. J. Dairy Sci., 84: 1413-1420.

Quinn, PJ; Carter, ME; Markey, BK and Carter, GR (1994). *Clinical veterinary microbiology*. 1st Edn., London, Wolf Publishing. PP: 21-66.

Rafyi-Barzoki, M (1998). Study of the prevalence of bacterial mastitis and the economic loss due to it in dairy farms in Semnan province. Final report of project, Ser. No. GN/60, Ministery of Jahad-e-Sazandegi. PP: 12-18 (In Persian with English abst.).

Siber, RL and Farnsworth, RJ (1981). Prevalence of chronic teat and lesions and their relationship to intramammary infection in 22 herds of dairy cattle. J. Am. Vet. Med. Assoc., 178: 1263-1267.

Souto, LI; Minagawa, CY; Telles, EO; Garbuglio, MA; Amaku, M; Melville, PA; Dias, RA; Sakata, ST and Benites, NR (2010). Correlation between mastitis occurrence and the count of microorganisms in bulk raw milk of bovine dairy herds in four selective culture media. J. Dairy Res., 77: 63-70.

Zamani, F; Babaei, M; Fazeli, MH; Sharifizadeh, A and Mohagheghpour, AR (2004). Economic study of subclinical mastitis in dairy herds in Isfahan, Iran. *Proceedings of the First Congress on Animal and Aquatic Sciences*. 31 August-2 September, Tehran, Iran. PP: 1022-1024.