WVJ
World's
Veterinary Journal

World's Vet. J. 3(1): 05-10, 2013

Original Article

Risk Factors Associated with Mastitis Occurrence in Dairy Herds in Benisuef, Egypt

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ABSTRACT

A cross sectional study was carried out from April, 2009 till the end of November, 2011 to estimate prevalence of mastitis and its risk indicators in private dairy herd in Beni-suef region. A total of 233 Holstein milking cows were tested using California Mastitis Test (CMT). Prevalence of mastitis at cow level was 42.92 % (100/233), out of which 9.87% (23/233) and 33.05% (77/233) were clinical and subclinical mastitis, respectively. The quarter level prevalence was 29.08 (272/929); from this the clinical and subclinical forms were 5.81 (54/929) and 23.47 % (218/929), respectively. Samples from all 54 active clinical cases and 98.0% (208/211) of positive CMT subclinical quarters were found to be culture positive. A total of 272 bacteria were isolated, the most prevalent being coagulase negative *Staphylococcus* (CNS; 37.8%), *S. aureus* (25.8%) followed by *E.coli* (18.7 %). Other bacterial isolates included *Streptoccusagalactiae* (11.8 %), *Klebsiellapneumonae* (3.6 %) and *Str.uberis* (2.8 %). Risk factors such as age difference, stage of lactation, parity, tick infestation, previous history of clinical mastitis, and farm hygiene were highly significant in the mastitis prevalence ($P < 0.01$). On the other hand, strong relationship was found between milk production and occurrence of bovine mastitis as, prevalence was higher in adult cows (X^2= 9.50, $P< 0.05$), hence the risk of developing mastitis significantly increase ($P< 0.003$) in lactating cow at ages (3-5 years) , at early lactation stage , with paritynumber (2-4) and during summer months, than those corresponding animals. In conclusion, the potential risk factors associated with mastitis prevalence and severity includes cow's itself and their surrounding environment particularly farm and milking hygiene procedure. Moreover, veterinary supervision, and tick infestation are among the potential risk factors predispose and increase severity of mastitis problem in dairy farm.

KEY WORDS: Bovine Mastitis; Prevalence; Risk Factors; Predisposing Factors, Tick Infestation.

INTRODUCTION

Mastitis remains the most common and the ambiguity disease of dairy cattle throughout most of the word. It continues to be the most economically important disease of dairy industry, accounting for about 38% of the total direct losses (Albenzio et al., 2002). Mastitis can affect any herd from the most organized to the least at any time; all herds are therefore potentially susceptible. Mastitis is a multifactorial disease, closely related to the production system and environment in which the cows are kept (Mekibib et al., 2010). Subclinical mastitis is responsible for the greatest financial losses associated with mastitis. It is estimated to cause 70% of the total losses. These losses however are difficult to demonstrate to producers since they are associated with decreased milk production caused by the effects of chronic inflammation of the mammary gland. Mastitis can be caused by a series of pathogens, differentiated into two broad categories: those causing contagious mastitis (S. aureus, Str. agalactiae, etc.) widespread from the infected quarters, primarily during milking (man hands, milking machines), and those causing environmental mastitis (Str. uberis, Str. dysgalactiae, coliforms, etc.) present in the environment (bedding, flooring, droppings) generally transmitted in any time of cow's life: during milking, between milking, during the dry period, especially at first calving, in heifers (Radostits et al., 2000). Monitoring udder health performance is impossible without reliable and affordable diagnostic methods (Zadoks and Schukken, 2006).

Thus, the most frequently used diagnostic methods are California Mastitis Test (CMT) and Bacteriological Culturing (BC) of milk. Epidemiological investigation of bovine mastitis, status of infection, treatment pattern would provide useful management information to the producer, veterinarian and other mastitis control team members (Shitandi et al., 2004). The present study was therefore designed to establish the prevalence and risk indicators of mastitis in dairy farm.

To cite this paper: Elbably, M.A., Emeash, H.H. and Asmaa. N.M. 2013. Risk factors associated with mastitis occurrence in dairy herds in Beni-Suef Governorate. *World's Vet. J.* 3(1): 05-10.
Journal homepage: http://wvj.science-line.com/

MATERIAL AND METHODS

Study area and period

A cross sectional study was conducted to precipitate the prevalence of mastitis in dairy herd, risk factors and in-vitro antibiotic profile in a private dairy farm in Beni-Suef region. The area is characterized by mild subtropical weather, with average minimum and maximum annual temperatures of 6.3 C and 38.6 C, respectively. The area also experience rain fall pattern with a short rainy season extending from December to February.

Study Animals and Husbandry Practices

A total of 233 Holstein dairy cows were kept in eight partially sheltered yards with earthy floors at stocking rate 12 m2/ cow. Lactating cows were milked three times / day in Herringbone milk parlour. CMT was applied once/month for few numbers of cows for detection of subclinically mastitic cows. Although all animals were routinely sprayed with insecticide (diazinon 0.1%) particularly at the beginning of spring and summer months, tick infestation was recorded throughout the study period. The hygienic measures prevailed were moderate.

Questionnaire survey

A structured questionnaire was prepared and information regarding to prevalence and risk indicators of mastitis in dairy herds including those attributed to both dairy cows and their farm .Cow attributes includes age, parity, stage of lactation, previous history of mastitis, tick infestation and other information related, while farm attributes includes farm hygiene & milking practices. Data related to causes of blindness were obtained from clinical records of the farm and interviews with the owner of the farm. The data were recorded for statistical analysis using Chi –square (X^2) test as the proportion of affected cows out of the total examined, (Systat Version, 1997).

Study Methodology

Detection of Mastitis: Mastitis was detected using the California Mastitis Test (CMT) and results of clinical inspection of the udder (Quinn 1999).

Physical examination of the udder: The udder was first examined visually and then through palpation to detect possible fibrosis, cardinal signs of inflammation, visible injury, tick infestation, atrophy of the tissue and swelling of the supra- mammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous health history of the mammary quarters and cause of blindness was obtained from case record sheets when available and by interviewing the farm owners when not. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions (Edmondson and Bramley, 2004).

California Mastitis Teat (CMT): Subclinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively (Harmon 1994). Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT according to Quinn et al. (1999). From each quarter of the udder, a squirt of milk sample was placed in each of the cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed well. Reactions were graded as 0 and Trace for negative, +1, +2 and +3 for positive.

Bacteriological examination of milk samples

Sampling methods: Samples were collected from lactating cows and their surrounding environments in examined farm. Animal samples were collected from teat apices & quarter milk samples from all lactating cows in examined farm. Teat apex swabs and individual quarter milk samples collected according to the procedures recommended by National Mastitis Council (1999). A representative samples from cow's surrounding including Milker's hands, teat cups of milking machines, water samples as described by according to (APHA, 1992).

Collection of milk samples: Before the collection of quarter milk samples from the tested cows, the udder was thoroughly cleaned with soap and water, rubbed dried and the teats were disinfected with cotton wool moistened with 70% ethyl alcohol, which is been allowed to be air dried. The first few squirts of milk were discarded. 5 - 20 ml of milk was collected in a sterile universal bottle. The quarter milk samples were kept in ice container and transported as soon as possible to the laboratory at the faculty of veterinary medicine, Beni-Suef University.

Culturing methods: The bacteriological culture was performed following the standard microbiological technique (Quinn et al., 1994). One loop full of milk was streaked on 5% sheep blood agar and MacConkey agar to detect bacteria that could grow on this medium. The plates were incubated aerobically at 37°C for 24 - 48 h. The plates were examined for growth, morphologic features of the colonies and hemolytic characteristic. Presumptive identification of bacteria on pure culture was done on the basis of colony morphology, heamolytic characteristics, Gram-stain and biochemical tests such as, coagulase test, heamolyses, pigment production, fermentation of maltose (purple agar +1% maltose).

Presence of Streptococcus spp. and Enterococcus spp. was determined according to CAMP reaction, type of heamolyses, growth characteristic on Edward's medium and sugar fermentation. Gram-negative isolates were identified based on growth on MacConkey agar, catalase test, oxidase reaction, triple sugar iron agar (TSI), IMVIC test, urease and sugar fermentation tests.

Identification of mastitis causing bacteria: Preliminary identification was done by observing the characheteristic morphology then by biochemical test according to Cruickshank et al. (1990).

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Biochemical Identification with commercial kit based methods (Himedia, India): The biochemical tests were performed with Himedia Identification kit which includes Voges-Proskauer reactions, phosphotase, ONPG, Urease production, arginine utilization, and 7 different carbohydrates utilization tests- Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose and Maltose. Further identification of isolates was examined with the standard chart (supplied by Himedia, India). Catalase test was performed according to a tube method using 3% Hydrogen peroxide. Appearance of bubbles confirmed presence of enzyme catalase.

Statistical analysis

Microsoft excel, 2003 and Stata 6.0 for windows 98/95/NT were used for data analysis. Descriptive statistics were used for all the variables. Chi-square (x^2) was used for assessing the statistical associations of various factors with mastitis.

RESUTS AND DISSCUION

The questionnaires survey revealed that, the average of clinical mastitis was 48 cases/ year, culling rate due to mastitis 10 cow's / year, clinically mastitic cows were only treated with systemic antibiotic, 14 quarters not respond to antibiotic therapy and developed chronic mastitis, no measures for detection of subclinically mastitic cows except CMT (once /month), shortage of milking hygiene procedure, tick infestation was recorded particularly in early spring and summer months. Saluiemi (1980) stated that current knowledge on the impact of the production environment on udder health is considerable. Moreover, practical experience of mastitis control has confirmed the importance of the stand structures, ventilation, milking machine, management practices, milking technique in particular and hygiene on udder health. Also Abdullah (2002) claimed that good management is the key factor on controlling the environment for protection and hence mastitis occurrence.

Table 1 revealed that prevalence of mastitis at cow's level was 42.92% (100/ 233), out of which 9.87% (23/233) and 33.05% (77/233) were clinical and subclinical, respectively. The quarter level prevalence was 29.28 (272/929); from this the clinical and subclinical forms were 5.81 (54/929) and 23.47% (218/929), respectively. These results are in accordance with those of Sori et al. (2005), De Schepper et al. (2006) meanwhile higher than, Biffa et al., (2005) recorded a lower incidence, 34.9% had mastitis, 11.9% clinical and 23.0% subclinical.

CMT score in relation to culture result of cow's quarters Table 2 indicated that the highest percentage of bacteriological positive quarters were had scores 2 &3+ (100 %) followed by Score +1 (98 %), while (0.8 & 0.53 %) with (- & ± resp.,). California mastitis test is a good for epidemiological survey of sub-clinical mastitis. Schukken et al. (1988) reported that CMT remains the only reliable screening test for detection of subclinical mastitis in dairy herds. Kapaga et al. (1995) concluded that CMT test is a good tool for epidemiological survey of sub-clinical mastitis in dairy herds.

Table 1. The prevalence and distribution of mastitis at cow's and quarters levels in examined farm

Distribution / Samples	Total (No.)	N.	SCM	CM	Total
		No. (%)	No. (%)	No. (%)	No. (%)
Cow's	233	133 (57.08)	77 (33.05)	23 (9.87)	100 (42.92)
Quarters	929*	657 (70.72)	218 (23.47)	54 (5.81)	272 (29.28)

N: Normal; SCM: Subclinical mastitis; CM: Clinical mastitis. *Other quarters were either dried or blind &excluded from the total examined number

Table 2. CMT score and culture result of cow's quarters in examined farm

Quarters / CMT result	Total (No.)	Culture (+ve)	
		No	%
(-)	574	3	0.53
(±)	90	7	0.8
(1+)	211	208	98.0
(2+)	6	6	100
(3+)	48	48	100
Total	929	272	29.28

(-): Negative; (±): Trace; (1^+): Week positive, (2^+): Distinct, (3^+): Strong positive.

Table 3 showed percentages of mastitogenic pathogens in different samples collected from from both cow's quarters and their surrounding environment in examined farm, it revealed that higher percentage of cow's teat apices were bacteriological positive (54.15 %) compared to 29.28 % of cultured quarter milk samples. meanwhile, the highest bacteria isolated from cow's environment was from bedding material collected from yards (61.4 %) followed by swabs from teat cups, Milker's hands (34.2 & 27.5 % resp.,) then water samples 7.5 %.These results indicated that Cow's environment particularly bedding , teat cups of milking machines and milker's hands represent a potential source of mastitis causing pathogens which attracted by teat apices of susceptible dairy cows. Saluiemi (1980) reported that if there is mastitis problem with cows in a loose house the cause is often poor milking hygiene or a faulty milking machine. Muddy outside pen or faulty ventilation, often combined with wet cubicles, which lead to mastitis problem caused by environmental pathogens (Radostits et al., 2000). Overall, poor hygiene may result in increased exposure and transmission of mastitis pathogens during milking.

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The prevalence of mastitis at cow's level as influenced by cow's age in Table 4 revealed that adult cows were more affected by subclinical mastitis 68 (33.83%) compared with clinical 19 (9.45%) respectively. These results substantiated that prevalence of mastitis was higher in adult cows compared with old cows may be due to bad hygienic condition during calving which are in harmony with that detected by Schroeder (1997), Bellamy (1999), Barkema et al. (1999). While the stage of lactation had a highly significant effect on prevalence of mastitis at (χ^2= 19.58, p<0.003**). Early stage and the period of involution of the mammary glands were the most susceptible stages with prevalence of (45.5%). These results are consistent with previous studies (Radostitis et al., 1994; Kivaria et al., 2007), similar to the result obtained by Biffa et al. (2005) and Pyroala (2002). Moreover, a highly significant influence of cow's parity on prevalence of mastitis in farm at (χ^2 = 80.17, p=0.000**). The highest percentage of mastitis was occurred in parities (2-4) (17.8, 26.7 & 8.7% resp.) followed by those of up to 2 parities (8.1, 34.76 & 10.16 % resp.,).

Table 3. The occurrence and distribution of mastitogenic pathogens in samples from cow's and their surrounding environment in examined farm.

Quarters / Samples	Total (No.)	Culture (+ve)	CNS	S. aureus	Str. agalactia	E.coli O157	E.coli O103	E.coli Total	Kl. pneumoniae	Str. uberis
Teat apices	460	54.15	37.30	13.89	-	21.82	5.95	27.77	6.35	12.69
Milk	929	29.28	37.8	25.2	11.8	9.7	8.9	18.6	3.6	2.8
Teat cups	120	34.2	46.34	17.1	19.5	12.2	-	2.43	2.43	-
Milker's hand	40	27.5	45.45	27.5	-	18.2	2.5	9.1	11.6	-
Water	40	7.5	33.3	33.3	-	-	33.3	-	33.3	-
Bedding	70	61.4	23.3	9.3	-	-	37.2	9.3	46.5	11.6

Table 4. The association between the occurrence of mastitis and various factors

Risk factors / Cow's	Examined (No.)	Bacteriologic (+ve) No.	Bacteriologic (+ve) %	SCM No.	SCM %	CM No.	CM %
Age (years)							
3-5	201	87	43.28	68	33.83[a]	19	9.45
6- 8	32	13	40.62	9	28.12[b]	4	12.50[a]
Lactation stage							
Early	233	106	45.5[a]	85	35.62[a]	21	8.15[b]
Mid	230	88	36.1[b]	61	26.52[c]	27	11.74[a]
Late	230	97	43.04[a]	76	33.04[b]	21	9.13[b]
Parity (No.)							
Up to 2	187	84	8.11[b]	65	34.76[a]	19	10.16[a]
2-4	46	16	17.78[a]	12	26.67[b]	4	8.69[b]
> 4	-	-	-	-	-	-	-
Season /year							
Autumn	238	42	17.65	35	14.7[c]	7	2.94[c]
Winter	220	70	31.82	57	25.91[b]	13	5.63[b]
Spring	231	73	31.60	60	25.97[b]	13	5.63[b]
Summer	240	87	36.25	66	27.50[a]	21	8.75[a]

Table 5. The association between the occurrence of mastitis and various risk factors

Factors	X²	P - value
Age	9.50	0.059*
Stage of lactation	19.58	0.003**
Parities	80.17	0.000**
Season of year	73.91	0.000**
Previous history of mastitis	173.08	0.000**
Tick infestation	70.95	0.000**
Cow's environment	160.87	0.000**

X^2 Chi square P - value = probability. * The difference was significant (p < 0.05). ** the difference was high significant (p < 0.01).

Season of year was found to exert a significant effect on prevalence of mastitis .Higher positive findings mostly contagious pathogens were recorded during summer and winter (36.25 % & 31.82% resp.) that was significantly at (P < 0.000**) different than those encountered in autumn and spring (17.65 %& 31.60 % resp) Which are in agreement with Fadlelmoula et al., (2007) and Matthews et al. (1992). The high frequency of mastitis during summer also followed by winter and spring may attributed to exposure of teats to a dirty environment, and teat lesions resulting from various causes, probably resulted in the increased intramammary infections.. These results are in agreement with those reported by Kivaria et al. (2007) and Fadlelmoula et al. (2007).

Risk factors such as age difference, stage of lactation, parities, tick infestation, previous history of clinical mastitis, farm hygiene (Table 5) were highly significant in the mastitis prevalence (P < 0.01), On the other hand, strong relationship was found between milk production and occurrence of bovine mastitis. (t - test = 51.32, P < 0.01) in Beni-Suef region, this result found to be agreed with Saluiemi (1980), who stated that the concentration of antibacterial factors in udder secretion are under genetic control and depend on the lactation stage and udder health. Moreover the teat canal represent a physical barrier to the penetration of bacteria when dilated, the risk of ascending infection is high. The teat canal remains open after milking for approximately 2 hr, in that time the cow may lie down during this critical period. Tick infestation and teat lesions findings were in contrast with the observation of Bekele and Molla (2001), who suggested that heavy tick infestation and teat lesions might be responsible for udder infection and also lead to udder abnormalities and deformities and blind in teats.

In conclusion; the nature of the numerous risk factors present on dairy farm studied, to varying degrees had a relationship with the prevalence of mastitis experience by cows on these farm. Lack of maintenance of strict hygiene and good sanitary environment may be a contributory factor in the cause of mastitis in the study area. It is therefore important that farmers should ensure strict personal hygiene, that of animals and general sanitary condition of the farm should be improved and maintenance

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