Epidemiological Studies on Subclinical Mastitis in Dairy cows in Assiut Governorate

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Abstract

In this investigation, some epidemiological studies were run on subclinical mastitis for totally 350 dairy cows of different breeds, ages and distributed in different villages in Assiut governorate, Assiut, Egypt, along a whole year (during the period from June 2006 till July 2007) through field screening surveys by using of the California mastitis test (CMT) for each quarter milk sample followed by bacteriological examination to identify the major causative agents of intramammary infection (IMI). The dairy cows were differed from the breed point of view as 230 Holstein Friesian breed and 120 native breed. Also, they were differed from the age point of view as a group of 95 cows aged from 2 to 4 years old and another group of 255 cow aged from 5 to 8 years old. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. All the cows lived nearly under the same conditions of breeding from the habitat, hygiene and feeding systems. The obtained results revealed that 67 cows (19.14%) had 80 infected quarters (5.71%). It was found that the most frequently major causative agents isolated were *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli* from the positive CMT samples with prevalence 52.5, 31.25 and 16.25%, respectively. With studying the breed factor, it was found Friesian breed was sensitive towards infection (20.43% at the cow level and 6.09% at the quarter level) than of native breed (16.67% at the cow level and 5% at the quarter level). It was also noticed that the prevalence of subclinical mastitis in hot weather as during summer (9.14% at the cow level and 2.64% at the quarter level) and during spring (4.86% at the cow level and 1.36% at the quarter level) was higher than in cold weather as during winter (2% at the cow level and 0.64% at the quarter level) and during autumn (3.14% at the cow level and 1.07% at the quarter level). In relation to age susceptibility, 5-8 years old cows (15.43% at the cow level and 4.36% at the quarter level) were susceptible than those of 2-4 years (3.71% at the cow level and 1.36% at the quarter level). The degree of quarter attack according to positive CMT was varied from 35 quarters (2.50%) showed degree (+++), to 45 ones (3.22%) showed degree (++), to 120 ones (8.57%) showed degree (+) and the rest (85.71%) showed degree (-). The obtained results threw the light on the epidemiology of subclinical mastitis in Assiut villages and provided an importance of the CMT for diagnosis of subclinical mastitis due to it is a reliable, easy, rapid and cheap tool helping in diagnosis and controlling the disease because it directs attention to individual mammary quarter that is secreting milk of high somatic cell content (SCC). Programs for control of subclinical mastitis may be planned around the routine examination of all lactating cows, and consequently early treatment can be applied towards positive cases rapidly for preventing their conversion towards clinical form among dairy cows and for protecting the herd health, milk hygiene and consequently the consumer health.

Key words: Epidemiology, Subclinical mastitis, California mastitis test (CMT), Intramammary infection, Dairy cows.

Introduction

Mastitis in both clinical and subclinical forms is a frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk (Harmon, 1994). Annual losses in the dairy industry due to mastitis was approximately 2 billions dollars in USA and 526 millions dollars in India, in which subclinical mastitis are responsible for approximately 70% of these dollars losses (Varshney and Naresh, 2004).

Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country (Ramachandrainh et al., 1990). Economical losses are due to (a) loss in milk production, (b) discarding abnormal milk and milk withheld from cows treated with antibiotics, (c) degrading of milk quality and price due to high bacterial or somatic cell count (SCC), (d) costs of drugs, (e) veterinary services and increased labor costs, (f) increased risk of subsequent mastitis, (g) herd replacement, and (h) problems related to antibiotics residues in milk and its products (Harmon, 1994; Barmely et al., 1996).

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk et al., 2003). Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing (Holdway, 1992). Although there are no visible or palpable external changes, the infection is present and inflammation is occurred in the udder (Blowey and Edmondson, 1995).

The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the Modified White Side test (MWT), SCC, pH, chlorine and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Lesile et al., 2002).

The CMT gives an indirect estimate of SCC because it based upon a gelling reaction between the nucleic acid of the cells and a detergent reagent. The CMT was chosen in several investigations because it is more perfect, efficient and reliable than other field and chemical tests for diagnosis of subclinical mastitis (Viani et al., 1990; Behera and Dwivedi, 1992; El-Balkemy et al., 1997). El-Attar et al. (2002) noticed that the CMT can detect more cases than MWT. While, Mahmoud (1988) mentioned that Schalm test was more reliable than other tests. Moreover, Park et al. (1982) reported that CMT was in a good agreement with bacteriological results.

Identifying and eliminating intramammary infection (IMI) may have significant economic benefits as preventing clinical mastitis and decreasing the amount of discarded milk (Dingwell et al., 2003). Additionally, knowledge of the clustering of IMI, both within quarters of a cow or within a herd, may be of considerable interest and may lead to further understanding of the dynamics of the disease (Lam et al., 1996).

Otherwise, from public health view, the assessment of subclinical mastitis etiological pathogens aids to classify the healthy sound milk samples from those of public health hazard as the limits recommended by European countries standards (IDF, 1996) and Egyptian standards (Egyptian Standards, 2001).

Because mastitis is one of the most costly and troublesome diseases in dairy cows in Egypt (Seleim et al., 2002) and also considered of vital importance in its association with many zoonotic diseases in which milk acts as a vehicle of pathogens causing tuberculosis and brucellosis (APHA, 1993; El-Balkamy et al., 1997; Shoshani et al., 2000) and because in its subclinical form a major part of the cow's udder has already been affected and the quality and quantity of milk reduced (Harmon, 1994), its detection in its subclinical form is very important.

Due to the aforementioned economic and public health importance, this study aimed (a) to elucidate the prevalence of subclinical mastitis in apparently healthy dairy cows in Assiut governorate, (b) to find out the most frequently major contagious causative agents causing IMI, and (c) to run some epidemiological studies and factors affecting on subclinical mastitis, in which, the epidemiology was defined as the study of disease in populations and of factors that determine its occurrence (Thrusfield, 2005).

Materials and Methods

Animals: A total of 350 dairy cows were examined in different villages in Assiut governorate, Assiut, Egypt, spread out over one year (during the period from June 2006 till July 2007). The dairy cows were differed according to breed as 230 Holstein Friesian breed and 120 native breed cows. Also, they were differed from the age point of view as a group of 95 cows aged from 2 to 4 years old and another group of 255 cow aged from 5 to 8 years old. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. They lived nearly under the same conditions of breeding from the habitat, hygiene and feeding systems. All animals were subjected to clinical and physical examinations with special interest towards the udder and teats. At the time of each examination, the breed of the cow, season, age of the cow, degree of quarter attack and the village site were recorded.

Milk samples: In a clean environment, thoroughly wiping the teats with 70% ethyl alcohol with paying extra attention to teat orifice was applied. After discarding the first few milk squirts, a total of 1400 individual quarter milk samples were subjected to the CMT.

CMT (Schalm et al., 1971): Each 3 ml of milk sample

Table-1. Relation between the positive CMT and the infection status of the quarters.

The CMT score	No. of the quarters	Bacterial isolates	%
3	35	26	74.29
2	45	39	86.67
1	120	15	12.50
Total	200	80	40.00

Table-2. Relation between subclinical mastitis and breed.

Breed	Cows			Quarters		
	Total	Infected	%	Total	Infected	%
Friesian	230	47	20.43	920	56	6.09
Native	120	20	16.67	480	24	5.00
Both	350	67	19.14	1400	80	5.71

from each quarter was drawn in each of the 4 shallow cups in the CMT paddle (US PAT NO. 2935384) then approximately equal volume of 3 ml of the commercial available CMT reagents (Original Schalm CMT Technivet, 4 industry road, Box 189 Brunswick, Maine 04011, USA) was added to each cup and mixed together through swirling the paddle in a circular motion for few seconds.

According to the visible reaction of the CMT, the results were classified into four scores: 0 = negative or traces (no change in consistency), 1 = slightly positive (+), 2 = positive (++) and 3 = highly positive (+++). Scores 1, 2 and 3 depend on the degree of gellation that were indicated by gelatinous mass (Schuppel and Schwope, 1998).

Bacteriological cultures: Each positive CMT milk sample was collected under aseptic conditions in a sterile screw caped bottle numbered to identify the particular quarter. All milk samples were sent directly to the laboratory with a minimum of delay for routine culture techniques.

Milk samples were cultured onto 10% sheep blood agar and MacConkey agar plates according to Carter and Cole (1990). Suspected colonies were identified morphologically, microscopically and biochemically according to Quinn et al. (1994) and Waage et al. (1999).

Results

1. Relation between the CMT and the infection status of the quarters: Out of the 1400 quarter milk samples examined by the CMT, 200 samples (14.29%) were positive. The results of bacteriological examination of these samples in relation to the recorded scores are shown in Table 1. Bacterial pathogens were found in 40% of the positive CMT samples (5.71% of all quarters). Bacterial analysis results showed 3 dominating bacterial species. They were Staphylococcus aureus, *Streptococcus agalactiae* and *Escherichia coli* isolated from 42, 25 and 13 quarters milk samples, respectively, in a percentage of 52.5, 31.25 and 16.25%.

2. Relation between subclinical mastitis and breed: From the 350 cows tested in this investigation, 67 cows (19.14%) had 80 quarters (5.71%) suffered from subclinical mastitis. Among the positive cases, 47 were of Friesian breed cows (20.43%) and 20 cows of native breed (16.67%) (Table 2). At the quarter level, also higher percentage of infected quarters in Friesian breed cows as 6.09% (56 out of 920 quarters) than those of native breed cows as 5% (24 out of 480 quarters).

3. Relation between subclinical mastitis and

Table-3. Relation between subclinical mastitis and season.

Season	Breed	Infected cows						Infected quarters					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Summer	Friesian	21	6.00	32	9.14	67	19.14	25	1.79	37	2.64	80	5.71
	Native	11	3.14					12	0.86				
Autumn	Friesian	8	2.29	11	3.14			11	0.79	15	1.07		
	Native	3	0.86					4	0.29				
Winter	Friesian	4	1.14	7	2.00			5	0.36	9	0.64		
	Native	3	0.86					4	0.29				
Spring	Friesian	14	4.00	17	4.86			15	1.07	19	1.36		
	Native	3	0.86					4	0.29				

Table-4. Relation between subclinical mastitis and age.

Age	No.	Breed	Cows			Quarters		
2-4 years	Total	Friesian	72			288		
	(95 cows)	Native	23			92		
	(380 quarters)							
	Infected	Friesian	Su*	3	8	Su	5	12
	(13 cows = 3.71%)		Au*	1		Au	2	
	(19 quarters = 1.36%)		Wi*	1		Wi	2	
			Sp*	3		Sp	3	
		Native	Su	1	5	Su	1	7
			Au	1		Au	1	
			Wi	1		Wi	2	
			Sp	2		Sp	3	
5-8 years	Total	Friesian	158			632		
	(255 cows)	Native	97			388		
	(1020 quarters)							
	Infected	Friesian	Su	18	39	Su	20	44
	(54 cows = 15.43%)		Au	7		Au	9	
	(61 quarters = 4.36%)		Wi	3		Wi	3	
			Sp	11		Sp	12	
		Native	Su	10	15	Su	11	17
			Au	2		Au	3	
			Wi	2		Wi	2	
			Sp	1		Sp	1	

Su* means summer, Au* means autumn, Wi* means winter and Sp* means spring.

season: With regard to mammary gland infection frequently according to season, the presented results in Table 3 showed the prevalence of subclinical mastitis in hot weather as during summer was 9.14% at the cow level and 2.64% at the quarter level and during spring was 4.86% at the cow level and 1.36% at the quarter level, while, in cold weather as during winter was 2% at the cow level and 0.64% at the quarter level and during autumn was 3.14% at the cow level and 1.07% at the quarter level.

The infected cows and quarters according to season were subdivided according to their breed as shown in Table 3.

4. Relation between subclinical mastitis and age: In a general way, the present results spread out over the year showed that among the 350 cows tested where 95 of them belonged to the age group of 2 to 4 years, which 13 cows (3.71%) had 19 infected quarters (1.36%). However, the remainder of the cows (n = 255) belonged to the age group of 5 to 8 years, of which 54 cows (15.43%) had 61 infected quarters (4.36%). The infected cows and quarters according to their age were subdivided according to breed and season as shown in Table 4.

5. Relation between positive CMT and degree of quarter attack: Results of positive CMT realized on quarters showed that 200 out of 1400 (14.29%) quarters were reached with variable degree of attack with subclinical mastitis. The degree was related to the CMT score as represented in Table 5. It was found that 35 out of the totally 1400 quarters (2.5%) showed degree (+++), 45 ones (3.22%) showed degree (++), 120 ones (8.57%) showed degree (+) and the rest (85.71%) showed degree (-). The relation of the degree of quarter attack with the other studied factors (breed, season and age) were recorded in Table 5.

Discussion

Mastitis in dairy cows is a serious problem as it is an economically devastating disease causing immense economic losses in the dairy industry in Egypt (Seleim et al., 2002) and is the worldwide costliest production disease in dairy herds (Miller et al., 1993). Subclinical mastitis is the most serious type as the infected animal shows no obvious symptoms and secrets apparently normal milk for a long time, during which causative organisms spread infection in herd, so it is an important feature of the epidemiology of many forms of bovine mastitis (Bakken and Gudding, 1982). The epidemiological studies applied in this investigation were applied through combination of the CMT with bacteriological cultures, why? Because subclinical mastitis was defined as when mammary glands without clinical abnormalities giving apparently normal milk but was bacteriologically positive and with positive CMT (Stefanakis et al., 1995). El-Balkemy et al. (1997) concluded the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable,

Table-5. Relation between subclinical mastitis and degree of quarter attack.

Degree	CMT Score	No. /1400 (%)	Breed	Total No.	Season				Age (years)	
					Su*	Au*	Wi*	Sp*	2-4	5-8
(+++)	3	35	Friesian	26	12	5	2	7	5	21
		(2.50)	Native	9	4	2	2	1	3	6
(++)	2	45	Friesian	31	16	5	3	7	6	25
		(3.22)	Native	14	6	3	2	3	5	9
(+)	1	120	Friesian	83	25	23	13	22	18	65
		(8.57)	Native	37	21	8	3	5	11	26
(-)	0	1200	Friesian	780					259	521
		(85.71)	Native	420					73	347

Su* means summer, Au* means autumn, Wi* means winter and Sp* means spring.

accurate and reliable method to confirm the causative organisms.

Additionally, many investigations (NMC, 1999; Dingwell et al., 2003; Milne et al., 2003) had assured that bacteriological culture is the gold standard method for identifying IMI but till nowadays the bacteriological sampling is not feasible as a routine test to identify subclinical mastitis. The indirect tests of mastitis seem to be more suitable for selecting cows with IMI (Pyorala, 2003). Also, Sargeant et al. (2001) added that logistic and financial considerations involved with sampling all fresh cows for bacteriological culture have precluded the widespread adoption of this strategy in the dairy industry. Over the past 50 years, many studies have been conducted in attempts to validate the CMT as a predictor of IMI. So, the CMT is a screening test for subclinical mastitis that can be used easily at cow-side (Leslie et al., 2002). It is widespread used in dairy fields and recommended (Shitandi and Kihumbu, 2004) and considered as rapid and characteristics indicator for the infection of mammary gland (Al-Anbari et al., 2006). Why in the present study investigators aimed to screening the subclinical form of mastitis? Because (a) the subclinical form is considered 15-40 times more prevalent than clinical form and accounts for greater losses in terms of milk production (Harmon, 1994), (b) it represents a reservoir of infectious organisms, and (c) it is related to approximately 70% of economic losses of mastitis (Varshney and Naresh, 2004).

From the aforementioned results presented in Table 1, the recorded overall quarter incidence of subclinical mastitis by the CMT was 14.29% (200 quarters out of totally 1400). Higher incidences were obtained by Coni et al. (1983); Alexandrova (1986); Mahmoud (1988); Ismail and Hatem (1998), in Italy, Bulgaria, Egypt and Saudi Arabia, respectively.

Major bacterial pathogens were recovered from 40% of positive CMT samples (5.71% of the quarters). A correlation between CMT and bacterial isolation has been previously demonstrated by Egan (1982) versus Fruganti and Valente (1980) who detected a low correlation between them. The positive CMT without isolation of the causative agent may attributed due to one of these 3 probable causes (a) failure to isolate organisms by the employed cultural techniques (selective media for mycoplasmas, haemophilus and fungi were not employed), this reason was in agreement with Ismail and Hatem (1998), (b) samples with unspecified mixed cultures were considered contaminated and thus excluded from subsequent analysis, and (c) certain percent 10-20% of cows sampled for bacterial culture based on CMT score will have no growth due to a number of factors including short lived infections that have been cleared by the cow or infections that are characterized by intermittent shedding of bacteria (*S. aglactiae, S. aureus, Mycoplasma*).

Bacteria that mostly frequently cause mastitis can be classified into two main categories; major and minor pathogens (Harmon, 1994; Sargeant et al., 2001). *S. aureus, S. agalactiae* and *E. coli* are the most common etiological agents involved in subclinical and clinical cases of mastitis in dairy cows (Gonzalo et al., 2002). While, *Pseudomonas aeroginosa, Corynebacterium pyogenes* and some *Aerobacter Spp*. are less common (Haynes, 1985).

Here, from the bacteriological cultures, the most frequently major contagious isolated pathogens were *S. aureus, S. agalactiae* and *E. coli,* isolated from 42, 25 and 13 quarters milk samples, respectively, in percentages of 52.5, 31.25 and 16.25%. About half of the previous percentages related to *S. aureus*, that because *S. aureus* is one of the most prevalent bacteria in subclinical mastitis in dairy cows and in our investigation may be return to milker hands which consider the main tool in distribution of microorganisms from teat to teat and from cow to cow, in addition to lack of hygiene (El-Balkemy et al., 1997). Radositis et al. (2000) divided the infective agents into (a) causes of contagious mastitis particularly *S. agalactiae* and *S. aureus*, and (b) causes of environmental mastitis e.g. *E. coli, Streptococcus dysgalactiae* and *Steptococcus uberis.* The isolated organisms were the 3 usual mastitis pathogens and were coincided with El-Balkemy et al. (1997); El-Attar et al. (2002); Dego and Tareke, (2003) because *Staphylococci* and *Streptococci* causes 90% of bovine mastitis (Poutrel, 1983).

As recorded in Table 2, it was noticed that 19.14% of the examined dairy cows was infected. 16% was detected by Makar (1997), While, higher incidences were achieved by Morcos et al. (1991) as 66.8%, Fandrejewska (1993) as 65.5%, Ghazi and Niar (2006) as 81.4%.

The factors affecting mastitis come into question? That is due to mastitis has a multifactorial nature that predominates with a clear interaction between host, agent and environment (Thrusfield, 2005). For this reason, the studied factors here were determined as hypothesized risk factors affecting mastitis as breed (Bendixen et al., 1988), season, age (Hultgren 2002), management, environment (McDougall, 2003) and hygiene (Ward et al., 2002).

With studying the breed factor (Table 2), it was found the native breed cows (16.67%) were resistant than the Friesian breed cows (20.43%). Our obtained result was in accordance with many studies applied also in Africa. In southern Ethiopia, Dego and Tareke, (2003) found indigenous Zebu more resistant than Holstein-Friesian. In kraals, Tanzania, Mdegela et al. (2005) found traditional animals more resistant than dairy animals. In Tiaret, Algeria, Ghazi and Niar (2006) found local breed more resistant compared to the imported ones. This is primarily due to the genetic resistance (Payne and Wilson 1999), and also to the bad adaptation of these cows to local environment and climate. However, more studies are needed to shed more light on this differential udder infection rates between local and imported breeds.

With regard to the influence of season (Table 3), it was noticed that subclinical mastitis frequency in the examined dairy cows was more significant in hot weather as during summer (9.14%) and during spring (4.86%) than in cold weather as during winter (2%) and during autumn (3.14%). These observations were explained from the complains of the farmers, in which, poor feeding, unbalanced rations and bad hygiene become more pronounced during the hot weather as the green fodders become deficient, in addition to, increasing the insect population. That led to decrease the immunity and consequently the mastitis increased. While during the cold weather, clover becomes somewhat enough and insect population becomes limited to spread infection.

Several authors reported an increase in mastitis frequency with age (Schultz, 1977; Dohoo et al., 1982; Bendixen et al., 1988). Our results in Table 4 were in agreement with those reported by these authors as 5-8 years old cows (15.43%) were susceptible to subclinical mastitis than those of 2-4 years (3.71%).

In the relation between subclinical mastitis and degree of quarter attack, results of positive CMT realized on quarters showed that 200 out of 1400 (14.29%) quarters were reached with variable degree of attack with subclinical mastitis. The results were summarized in Table 5 showing 2.50, 3.22 and 8.57% of quarters gave (+++, ++ and +) degrees, respectively. Attia et al. (2003) found 30 and 75% gave (+++ and ++) degrees, respectively.

Our results that presented in Tables from 1 to 5, focused on the numbers of the infected quarters in parallel to the infected cows. If measurements taken at cow level with ignoring quarters level, serious underestimation of treatment effects in such affected quarters (Barkema et al., 1997). Additionally, transmission of IMI occurs not only among cows but also among quarters within a cow (Lam et al., 1997).

This work studied the prevalence of subclinical mastitis accompanied with analysis of different factors including breeding distribution, different seasons, different ages and degree of quarter attack, and the following elements are considered: Friesian breed was more struck by subclinical mastitis than native one, youngest cows had the most sensitivity and hot weather was with high prevalence.

The obtained prevalence may be attributed to a group of shared factors of breeding where the dairy cows lived, including bad habitat, lack of hygiene, unbalanced food and bad draft. This group of defective conditions played a role in rendering the udder more susceptible to IMI (Ghazi and Niar, 2006). On the other hand, good management practices such as milker hygiene, sanitization of milking machine and udder healthy environment as well as dry off treatment and controlling other predisposing diseases should be considered among the major prophylactic measures to minimize the occurrence of the disease. Furthermore, all dairy producers know that early detection of IMI is important for selecting and implementing proper therapy. Unfortunately, most infections are not detected until they become clinical, and by then extensive and costly damage can result. It was concluded the CMT can be used to monitor udder health trends over time and combined with culturing milk from the bulk tank, individual quarter or cow, groups or categories of cows adds an additional dimension to evaluating udder health and mastitis control programs. This information not only provides a snap-shot in time of the udder health situation of a herd, it provides a very effective means of plotting of identifying infection trends, identifying herd risk factors and monitoring herd performance and management interventions. Routine milk cultures should be an ongoing part of any mastitis control program. The sampling strategies for any ongoing program require the input of the herd veterinarian as well as herd management. It is particularly important that milkers be aware of any standard procedures for identifying cows to sample as well as appropriate sampling procedures.

In a spite of a large research efforts aimed to gain epidemiological knowledge and to develop a new control tools for mastitis, the clinical occurrence of this disease remains a substantial problem for dairy producers.

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