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## Prevalence of Subclinical Mastitis in some Dairy Cattle Farms in Kuwait

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### ABSTRACT

A survey to prevalence of subclinical mastitis (SCM) in some dairy cattle farms in state of Kuwait was carried out. 200 quarter milk samples from apparently healthy dairy cows. 200 quarter milk samples from apparently healthy dairy cows at Kuwait were investigated for subclinical mastitis using California mastitis test (CMT), Somatic cell count (SCC) and bacteriological isolation. The results revealed that the prevalence of subclinical mastitis was 45 % and bacterial culture was positive in 37.5%. SCC was assessed in 200 milk samples, the minimum and maximum SCC was  $0.12 \times 10^5$  cell/ ml and  $4.75 \times 10^5$  cell/ ml with the mean of  $1.7 \pm 18.11 \times 10^5$  cell/ ml. The cultural examinations to determine subclinical mastitis causative agents revealed that the isolated bacterial strains in examined milk samples of cows were *Staphylococcus aureus*, *Streptococcus agalactia*, *E. coli* and *Pseudomonas aeruginosa* with distribution of 37.6%, 20.5%, 19.4% and 3.2%, respectively as single infection from total 93 isolated bacterial strains. Meantime, there were mixed infection of (*Staphylococcus aureus* + *E. coli*); (*Streptococcus agalactia* + *E. coli*) and (*Staphylococcus aureus* + *Pseudomonas aeruginosa*) with the recovery rates of distribution of 8.6%, 9.7%, and 1%, respectively. Antibiogram profile of some representative field strains of *Staphylococcus aureus*, *Streptococcus agalactia*, *E. coli* and *Pseudomonas aeruginosa* isolated from subclinical mastitis cases to 11 different antibiotics. It revealed that all *Staphylococcus aureus*, *Streptococcus agalactia*, *E. coli* and *Pseudomonas aeruginosa* isolates were highly sensitive to enrofloxacin, and Cloxacillin. However, it showed variable sensitivity degrees to other tested antibiotics. It could be concluded that subclinical mastitis constitutes a major economic and healthy problem for dairy herd in Kuwait.

**Keywords:** Subclinical mastitis, dairy cattle, Kuwait, bacterial causes, antibiogram.

### 1. INTRODUCTION

Milk is an important diet of human beings. It contains a wide range of dietary constituents of vital importance like water, proteins, lactose, minerals and vitamins (Allore, 1993). Subclinical mastitis (SCM) is an economically important disease of dairy cows and has ranks amongst the factors that limit milk production. It has a tendency to persist because usually it remains unobserved which results in high milk somatic cell count (SCC) and reduced milk production, treatment cost and development of clinical mastitis which leads to heavy economical losses (Leitner *et al.*, 2011). Total milk loss from quarters affected with subclinical mastitis has been estimated to vary from 10% to 26% and approximately 75% of the economic loss from subclinical mastitis is attributable to loss of milk production (Radostits *et al.*, (2000). Milk from the affected cows that contaminated with bacteria makes it unhealthy for human consumption and has a zoonotic importance (Sharif *et al.* (2009).

Subclinical mastitis is a great challenge among the dairy farmers as it is indirect and more difficult to detect due to the absence of any visible symptoms and needs the availability of a rapid screening test for the early detection of the disease (Viguier *et al.*, 2009). It is characterized by reduced host defense mechanism (Owen *et al.*, 2000), as well as alterations in some enzymes and metabolites (El- Zubeir *et al.*, 2005; Andrei *et al.*, 2009 and Matei *et al.*, 2010).

The most significant subclinical abnormality of the milk is the increase in somatic cell counts, the most common measure of milk quality and udder health (Radostits *et al.*, 2000). Milk and other dairy products that obtained from the subclinical mastitis cases are commonly contaminated with *Staphylococcus aureus* and *Streptococcus agalactiae* (Hameed *et al.* (2006). Milk from cows with subclinical mastitis accidentally mixed into bulk milk enters food chain and poses a hazard to human health.

Contagious mastitis pathogens are generally spread from cow-to-cow with the infected udder being the primary source of the pathogen, via milk-contaminated fomites at milking, milkers and by milking machine (Awandkar *et al.*, 2009), while environmental mastitis pathogens are found in the environment where the cow lives and spread directly from there to the udder and transmitted by contact of teats with contaminated soil, bedding and water with fecal materials (Mekonnen and Tesfaye, 2010).

Various methods of detection of subclinical mastitis have been found including California Mastitis Test (CMT) which is considered a better and a rapid indirect diagnostic tool to detect subclinical mastitis (Zaki *et al.*, 2008). Also, measurement of somatic cell count and detection of the causative microorganisms (Viguiet *et al.*, 2009). As well as bacteriological culture of milk samples served as a gold standard method for assessment of different tests used for diagnosis of subclinical mastitis and evaluation of intramammary infection (National Mastitis council, 1999). Antibioassay assessment of isolated bacteria is necessary tool for treatment and tracing the emergence of multi-drug resistance strain of such isolated bacteria. Many authors evaluated the sensitivity of isolated bacteria from subclinical mastitis against different antibiotics (Denamiel *et al.*, 2005; Idriss *et al.*, 2014 and Singh *et al.*, 2018).

Due to the economic and public health importance of subclinical mastitis, the aim of this work was to determine prevalence of subclinical mastitis and associated causative bacteria, the association of SCC and occurrence of bacteria with SCM in Kuwait dairy farms was evaluated. Also, antibiogram assessment of isolated bacteria was performed.

## 2. MATERIALS AND METHODS

### 2.1. Milk sampling:

200 quarter milk samples were collected from each quarter of 200 randomly selected apparently healthy lactating cows were collected from different dairy farms in state of Kuwait for detection of subclinical mastitis during the period from April till the end of September 2017. Samples transferred to the laboratory in ice-box to maintain its chemical and microbiological status unchangeable (Radostits *et al.*, 2007). All animals had no evidence of apparently healthy at the time of sampling. Each sample was mixed thoroughly before being divided into two parts. The first was screening by CMT and SCC, and the second for bacteriological examinations.

### 2.2. Detection of subclinical mastitis:

200 quarter milk samples were tested for subclinically mastitic cows by screening California mastitis test according to Schalm and Noorlander (1957).

### 2.3. Somatic cell count:

Collected quarter milk samples were examined for SCC according to Zecconi *et al.* (2002). By using automatically Bentley Soma count, 150 (Bentley U.S.A). The sample was warmed in water bath at 40°C for 5 minutes then mixed automatically before automatic reading of SCC by Bentley Soma count 150 (Bentley U.S.A.) for dispersion of fat globules. The somatic cell count measures the number of white blood cells including neutrophils, macrophages, lymphocytes, eosinophils and various epithelial cell types of the mammary gland in milk that were present in large number in case of subclinical mastitis.

### 2.4. Bacteriological examination of milk samples:

#### 2.4.1. Preparation and cultivation of milk samples:

Fresh milk samples were incubated aerobically at 37°C for 24-48 h., then loopfulls of incubated milk were streaked onto plates of blood agar (for detection of hemolysis), Mannitol salt agar (selective media for *Staphylococci*), Edward's media (selective media for *Streptococci*) and Eosine Methylene blue (EMB) agar (selective media for *E. coli*). *Pseudomonas* selective agar (selective media for *Pseudomonas aeruginosa*). The suspected colonies were further identification according to Quinn *et al.* (2002) and Collee *et al.* (1989).

#### 2.4.2. Antibiogram assessment of isolated bacterial strains from subclinical mastitic milk:

Antibiogram profile of some representative field strains of *Staphylococcus aureus* (25), *Streptococcus agalactia* (15), *E. coli* (15), with *E. coli* and *Pseudomonas aeruginosa* (3) isolated from subclinical mastitis cases isolated from mastitic cows are purified and identified biochemically and tested in vitro for their sensitivities to 11 different types of antibiotics and commonly used in veterinary practical field for mastitis prevention and control were used in the present work which including; Penicillin G (10 µg), gentamycin (10 µg), enrofloxacin (10 µg), Cloxacillin (30 µg), Streptomycin (10 µg), colistin (25 µg), neomycin (30 µg), erythromycin (15 µg), kanamycin (30 µg), amoxicillin (30 µg) and ampicillin (30 µg). using standard procedures as described by Quinn *et al.* (1994).

## 3. RESULTS AND DISCUSSION

Subclinical mastitis is a disease in which there is no detectable inflammatory change in the udder and no

observable abnormalities in the milk. However, there are many cellular, biochemical, immunological and enzymatic changes are present in milk from udder with subclinical infection. These changes are detected by laboratory tests such as screening and routine tests applied for diagnosis of subclinical mastitic cases. Subclinical mastitis is more prevalent than the clinical form, so, it causes great losses in the dairy herds (Joshi and Gokhale, 2004). Also, subclinical mastitis usually develops the clinical form, it reduces milk production and adversely affect milk quality. Total milk loss from quarters affected with subclinical mastitis has been estimated to vary from 10% to 26%. Approximately 75% of the economic loss from Subclinical mastitis is attributable to loss of milk production. The most significant subclinical abnormality of the milk is the increase in somatic cell count, which is the most common laboratory way of milk quality and udder health (Sharif et al. (2009).

Table (1): Results of SCM and bacteriological culture in mastitic cow

Parameters	No. of Quarters samples	Positive samples	
		No.	%
SCM positive (SCC >= 1x 10 <sup>5</sup> cells/ml)	200	90	45
Bacteriological culture		75	37.5

Results summarized in Table (1) showed that by the investigating a total of 200 cow's quarter milk samples using California mastitis test and bacteriological isolation revealed that with prevalence of subclinical mastitis was 45 % (90 out of 200) and bacterial culture was positive in 37.5% (75 were positive out 200) Nearly similar findings were previously reported by many authors (Bitew et al., 2010; Girma, 2010 and Mir et al., 2014) who reported prevalence of 32.4, 34.4 and 30.73%, respectively. However, higher prevalence was cited by many investigators (Zeryehun et al., 2013; Abrahmsen et al., 2014 and Zenebe et al., 2014). On the other hand lower prevalence was previously reported by many workers (Biffa et al., 2005; Alemu et al., 2013 and Zizet, 2015).

In the present study, the somatic cell count was assessed in 200 milk samples. As shown in table (2), the minimum and maximum SCC was 0.12 x 10<sup>5</sup> cell/ ml and 4.75 x 10<sup>5</sup> cell/ ml with the mean of 1.7±18.11 x 10<sup>5</sup> cell/ ml. Milk SCC has been extensively as indicators of intramammary infection (IMI), Somatic cell count has been included as a component of the definition of mastitis and the original limit for SCC of a healthy quarter is 500.000 cell/ml. The definition

Table (1): Prevalence of Subclinical mastitis and status of quarters analyzed Bacteriologically Parameter.

Was a guide for diagnosis, even though 50% of truly infected quarters could at any time have a cell count less than the break-point of 500.000 cell/ml (International Dairy Federation (IDF: 1971). SCC is a useful index for detection of subclinical mastitis and milk quality. The SCC of normal milk is less than 200.000 cells/ml, higher SCC is considered as abnormality in milk and indicates udder infections. High SCC causes a rise in whey protein and a decrease in casein, resulting in a considerably lower cheese yields. Shorter shelf life and adverse milk flavor are other consequences of high SCC (Bilal and Ahmed, 2004)

Table (2): Mean SCC of subclinical mastitic mammary quarters

No. of examined samples	SCC (x 10 <sup>4</sup> cells/ml)		
	Minimum	Maximum	(Mean ± SEM)
200	0.12	4.75	1.7±18.11

Results summarized in Table (3) showed that frequency distribution of bacteriological isolates recovered from subclinical mastitic milk of 200 samples of dairy cows at quarter level in state of Kuwait. The cultural examinations to determine subclinical mastitis causative agents revealed that the isolated bacterial strains in examined (200) milk samples of cows were *Staphylococcus aureus*, *Streptococcus agalactia*, *E. coli* and *Pseudomonas aeruginosa* with distribution of 37.6%, 20.5%, 19.4% and 3.2%, respectively as single infection from total 93 isolated bacterial strains. Meantime the results proved that there were mixed infection of (*Staphylococcus aureus* + *E. coli*); (*Streptococcus agalactia* + *E. coli*) and (*Staphylococcus aureus* + *Pseudomonas aeruginosa*) with the recovery rates of distribution of 8.6%, 9.7%, and 1%, respectively as mixed infection from total 93 isolated bacterial strains. These bacterial infections were previously isolated by Shereen (2014). The results of the present study concluded that *Staphylococcus aureus* was the most prevalent isolate. Similar incidence of staphylococci in cows was reported by Mir et al. (2014). A lower incidence of *Staphylococcus species* in cows was reported by Hanan et al (2015). Moreover, a higher percentage (77.2%) and (63.6%) of *Staphylococcus species* in cows was reported by Mekibib et al. (2010) and Elbably et al. (2013).

The high incidence of *Staphylococcus aureus* in case of subclinical mastitis may due to the wide spread use of antibiotics especially dipping teats in sanitizers (Jones and Swisher, 1998). The obtained results clarified that the high incidence of *Streptococcus agalactia* in cases of subclinical mastitis was due to the increase in the infection rates by *Streptococcus species*. This finding was fully supported by Kumar et al. (2014).

In the present study, *E. coli* prevalence as a causative agent of mastitis was 19.4%. Similar incidence (18.7%) of *E. coli* in cows was reported by Elbably *et al.* (2013). However, a lower result (4.6%) of *E. coli* in milk of cows was previously reported by Mekibib *et al.* (2010). Furthermore, a higher incidence (46.32) of *E. coli* in cows were reported by Hamdy (2009). *E. coli* is a frequent cause of bovine mastitis and widely distributed in nature emphasized that *E. coli* serotypes in mastitis milk were similar to fecal isolates. The incidence of *E. coli* mastitis in the present study may be attributed to many risk factors associated with housing, such as dietary changes or may be due to the heavy contamination of bedding and poor hygienic measures which cause a consequent udder infection from fecal contamination on the bedding (Robertson *et al.*, 1994). From the results achieved in Table (3), one could be noticed the most mixed infection was *Staphylococcus aureus* with *E. coli* followed by *Streptococcus agalactia* with *E. coli*. These data were in agreement of results of Dalia (2015).

Table (3): Frequency distribution of isolates from subclinical mastitis.

In the present study the frequency distribution of isolates and mean SCC of subclinical mastitic quarters according to the types of bacteria causing SCM in dairy cows was determined. The results achieved in Table (4) proved that there are a correlation between the infection of quarter and type of isolated bacteria such as single infection with *Pseudomonas aeruginosa* and *Streptococcus agalactia*, the mean SCC  $\pm$  SEM were  $3.8 \pm 0.96 \times 10^5$  and  $2.8 \pm 1.09 \times 10^5$ , followed single infection with *E. coli* and *Staphylococcus aureus*, the mean SCC  $\pm$  SEM were  $2.2 \pm 1.2 \times 10^5$  and  $1.33 \pm 0.97 \times 10^5$ . However; in mixed infection with *Streptococcus agalactia* and *E. coli*, the mean SCC  $\pm$  SEM were highest one ( $4.2 \pm 1.7 \times 10^5$ ). However, mixed infection of *Staphylococcus aureus* with *Pseudomonas aeruginosa*, the mean SCC  $\pm$  SEM were  $1.8 \pm 0.33 \times 10^5$ . The last one of mixed infection of *Staphylococcus aureus* with *E. coli*, the mean SCC  $\pm$  SEM were  $1.1 \pm 0.33 \times 10^4$ . It proved that *Pseudomonas aeruginosa* is organism must be not ignored as causative of subclinical mastitis. Moreover, the significant role of mixed infection of *Streptococcus agalactia* and *E. coli* the most potential organisms as causative of subclinical mastitis and of economic impact with public health significance as a cause of many neonatal infection and digestive and urogenital disorders in human consumers of such subclinically mastitic milk. This correlation to the type of bacterial isolates from the examined subclinical milk samples of cows come in agreement with the results of Bytyqi *et al.* (2010) who found that the environmental organisms produced less SCC than highly contagious ones. The result in Table (4) concluded that the mixed bacterial infections of subclinical mastitis showed higher SCC, This conviction was fully supported by the results of Zeinhom *et al.* (2013) who reported the mixed bacterial infections in dairy cows had higher level of SCC than single

infections. It could be noticed from the results achieved in Tables (1 to 4), that subclinical mastitis was more prevalent in examined cows in Kuwait. CMT and SCC are good laboratory tests for evaluation of subclinical mastitis in cows.

Antibiogram profile of some representative field strains of *Staphylococcus aureus* (25), *Streptococcus agalactia* (15), *E. coli* (15), with *E. coli* and *Pseudomonas aeruginosa* (3) isolated from subclinical mastitis cases to 11 different antibiotics commonly used in mastitis prevention and control were used the present work including; Penicillin G (10  $\mu$ g), gentamycin (10  $\mu$ g), enrofloxacin (10  $\mu$ g), Cloxacillin (30  $\mu$ g), Streptomycin (10  $\mu$ g), colistin (25  $\mu$ g), neomycin (30  $\mu$ g), erythromycin (15  $\mu$ g), kanamycin (30  $\mu$ g), amoxicillin (30  $\mu$ g) and ampicillin (30  $\mu$ g). The results in Table (5), revealed that all *Staphylococcus aureus* isolates (25) were highly sensitive to enrofloxacin, Cloxacillin and kanamycin, however, it showed sensitive to gentamycin

	Bacterial isolates	Cows at quarter level	
		No.	(%)
Single isolates	<i>Staphylococcus aureus</i>	35	37.6
	<i>Streptococcus agalactia</i>	19	20.5
	<i>E.coli</i>	18	19.4
	<i>Pseudomonas aeruginosa.</i>	3	3.2
Mixed isolates	<i>Staphaureus+ E.coli</i>	8	8.6
	<i>Streptagalactia+E.coli</i>	9	9.7
	<i>Staph. aureus+Ps. aeruginosa</i>	1	1
Total		93	100

and neomycin. Moreover, it was intermediate to Streptomycin. On the other hand all *Staphylococcus aureus* isolates were resistant to penicillin-G, ampicillin, colistin, erythromycin and amoxicillin. Table (5) also proved that all *Streptococcus agalactia* (15) were highly sensitive to enrofloxacin, Cloxacillin and gentamycin, however, it showed sensitive to neomycin. Moreover, it was intermediate to kanamycin and Streptomycin. On the other hand all *Streptococcus agalactia* isolates were resistant to penicillin-G, ampicillin, colistin, erythromycin and amoxicillin. Moreover, Table (5) also proved that all *E. coli* (15), were highly sensitive to enrofloxacin, however, it showed sensitive to Cloxacillin, kanamycin, penicillin-G, and ampicillin, erythromycin and Streptomycin. Moreover, it was intermediate to gentamycin, and neomycin. On the other hand all *E. coli* isolates were resistant to colistin and amoxicillin. Furthermore, the results in table (5) clarified that all *Pseudomonas aeruginosa* isolates (3) were highly sensitive to enrofloxacin, and gentamycin however, it showed sensitive to Cloxacillin, kanamycin, erythromycin and Streptomycin. Moreover, it was intermediate to penicillin-G, ampicillin and neomycin. On the other hand all *Pseudomonas aeruginosa* isolates were resistant to colistin, and amoxicillin.

**Table 4.** Frequency distribution of isolates and mean SCC of subclinical mastitic quarters according to the types of bacteria causing SCM in dairy cows Bacterial isolates

Bacterial isolates tested	Antibiotic disc	Staph. aureus (25)	Streptagalactiae (15)	Escherichia coli (15)	Pseudomonas aeruginosa (3)
Enrofloxacin	(10 µg)	+++	+++	+++	+++
Cloxacillin	(30 µg)	+++	+++	++	++
Gentamycin	(10 µg)	++	+++	+	+++
Kanamycin	(30 µg)	+++	+	++	++
penicillin-G	(10 µg)	-	-	++	+
Ampicillin	(30 µg)	-	-	++	+
Streptomycin	(10 µg)	+	+	++	++
Colistin	(25 µg)	-	-	-	-
Erythromycin	(15 µg)	-	-	++	++
Amoxicillin	(30 µg)	-	-	-	-
Neomycin	(30 µg)	++	++	+	+

The present study were coincided the results of many investigators and researchers as Bhattacharya *et al.* (2002) found that most of staphylococcal and streptococcal isolates from cows suffering from clinical and subclinical mastitis were highly sensitive to ciprofloxacin, tetracycline and gentamycin, less susceptible to penicillin, ampicillin and cloxacillin. Denamiel *et al.* (2005) found streptococcal strains, *Strept. agalactiae* and *Strept. dysgalactiae* strains isolated from bovine intramammary infections in Argentina were resistance to erythromycin and clindamycin but no isolates were resistance to penicillin G. Suriyasathaporn (2011) found that *S. aureus*, *S. dysgalactiae* and CNS were susceptible to most examined antibiotics. The present result also agrees with Idriss *et al.* (2014) who found most of the isolated pathogens were sensitive to enrofloxacin. Furthermore, Singh *et al.* (2018) reported that antibiogram for *Streptococcus agalactiae* revealed the highest sensitivity to ofloxacin, ciprofloxacin, gentamicin and resistance to amoxicillin and doxycycline; *Staphylococcus aureus* isolates were showed the highest

sensitivity to ciprofloxacin, doxycycline, azithromycin and sparfloxacin and resistance to amoxycillin and gentamicin. Similarly, *E. coli* revealed the highest sensitivity to

Bacterial isolates	No. of quarter	SCC (x10 <sup>5</sup> cells/ml) (Mean ± SEM)
<i>Staphylococcus aureus</i>	35	1.33±0.97
<i>Strept. Agalactiae</i>	19	2.8± 1.09.
<i>Escherichia coli</i> .	18	2.2± 1.2
<i>Pseudomonas aeruginosa</i>	3	3.8± .96
<i>Staphylococcus aureus</i> + <i>E. coli</i>	8	1.1± .33
<i>Strept. agalactiae</i> + <i>E.coli</i>	9	4.2± 1.7
<i>Staph.aureus</i> + <i>Ps. aeruginosa</i>	1	1.8± .33

azithromycin and chloramphenicol and resistance to amoxicillin and teicoplanin.

**Table (5):** Antibiotic sensitivities of bacterial isolates from mastitic cows.

(+++): Highly Sensitive; (++) : Sensitive; (+): Intermediate; (-): Resistant

**Conclusion**

In conclusion, our study suggested that using MOS as prebiotic; potentially improved growth performance parameters, survival rates; besides the improvement of gut morphology and microbiota that could be beneficial to fish health. Taken together these results, we conclude that however, treatment with the prebiotic, MOS, in feed and water, could be used to enhance the measured parameters of tilapia, using MOS as dietary additives will be more applicable and economic in feed than in both water and feed.

**Conflict of interest**

Authors declare that they have no conflict of interest

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