

# Introduction

The mutual name for the long-necked, humped , even-toed ungulates large animal is camel. It is including the mammalian genus *Camelus* of the *Camelidae* family. According to FAO ,the whole camels number internationally is supposed to be 20 million. The majority of these camels about 15 million are found in Africa and the rested in Asia. Because most of these camels are possessed by Bedouin (nomads), who travel along the seasons of the year in exploration of grassland, therefore the actual number of camels can only be predictable ([http://www.fao.org/ag/againfo/home/en/news\\_archive/2006\\_camel.html](http://www.fao.org/ag/againfo/home/en/news_archive/2006_camel.html)) .

The camel ( *Camelus dromedarius*) considered as an essential existence factor in the arid and semiarid areas of Middle East and Africa (Fo *et al.*, 2012). The camelids are separated from ruminants and located in the order of *Artiodactyla* that are a polygastric animals but not a true ruminant depending on taxonomy, physiology and behavior. Moreover, true ruminants own 4 part stomach, while, camel ruminates after feeding ,but has 3 part stomach , consequently, it is named a distinct as a pseudo-ruminant (Fowler, 1996). There are variation between the susceptibility of the Camelids and ruminants to infectious diseases. According to Canadian researchers during the outbreaks of bovine spongiform encephalitis that diagnosed in cows in Alberta/ Canada, any of transmissible spongiform encephalopathies haven't ever been investigated in Camelids in the globe (Fowler, 2010).

The old world camels place fundamentally into two species, the *Camelus bacterianus* (Bactrian) with two humps and *Camelus dromedarius* (Arabian) with one hump (Wilson, 1998). The dromedary favour desert environments and are used in the transportation of human and also as a source of hair, hides, meat and milk (Al-Salihi, 2016).

The Camelids are considered as one of the domesticated animals in Mesopotamia and this fact has been confirmed in the cylinder seals that came from Mesopotamia Middle Bronze Age and showed riders seated upon camels (Al-Salihi, 2016). According to Iraqi government survey in 1978, there was 70,000 camels. But this number dropped dramatically because of the economic sanctions imposed after the 1991 Gulf war (FAO, 2005). Nowadays, Iraq owned a total of 58,000 camels (Al-Salihi, 2012), all are one-humped camels and are commonly found in certain parts. The greatest proportion of this population is present in the middle and south and west parts of country. Throughout Iraq various ecozones desert areas are living the “Bedouin” communities, who own the camels and consume

their milk and meat. Moreover, camels are used for packing, transport, riding and production of leather and wool.

Camel is considered as one of the highly mulch animals, although they are living in the harsh desert environmental conditions (Knoess, 1984; Abbas and Tilley, 1990; Schwartz, 1992). The variation of milking frequency is one of the obstacle in the estimation of the camel's daily milk production within the pastoralist circumstances. Management environments, feeding, stage of lactation, breed, stage, species and diseases of the udder are the factors that affected on the milk production of the she-camels. She camels also show variation in the lactation length from 9 to 18 months.

Inflammation of the udder is called a mastitis. It is a compound disease that occurs in globe between a dairy animals. It causes huge economic losses due to drop of milk production, poor milk quality and extra cost from the treatment and care of infected udder (Maichomo *et al.*, 2011; Sudhan and Sharma, 2010; Eyassu and Bekele, 2010 ; AL-Ani , 2004). Mastitis has both impact of economic importance and as zoonotic disease that cause of numerous harmful effects on the health of human and animal production (Hegazy *et al.*, 2004 and Al-Majali *et al.*, 2008).

Mastitis is caused by different causative agents such as virus, bacteria and fungus, however, bacterial infections are considered as the primary cause of domestic animals mastitis (Eyassu and Bekele, 2010). She-camels is not commonly affected with mastitis, and if it occurs, it is similar to the forms that seen in dairy cattle , called clinical and subclinical. It also can lead to loss of function in one or more quarters or even death. There are different predisposing factors that enhance prevalence of mastitis. Anatomically, mammary glands of the she-camels are not pendulous accompanied with relatively short teats that lead to reduce the risk of the trauma. Even so, the possibility of laceration, contusion and abrasion can happen if she-camels attack by dogs or jump on tree or fence. The traumatized teat acts to wane the canal sphincter that in normal animals hinder the accessing of pathogenic microorganisms. Milk itself consider as an exceptional medium for growth of bacteria. If the milk is stagnating in the udder for any reason, this condition act to help in the growing od the invaded bacteria, settle and develop of mastitis.

Wernery *et al.*, (2008) reported the particular bacteria which cause mastitis in camelids. However, all cattle mastitis micoorganisms can predicated to be as the cause in she-camels, as these micoorganisms have also been investigated from other disease conditions in camelids. *Escherichia coli*, *Klebsiella pneumoniae*, and *Aerobacter enterobacterium* , have been isolated from camelids peracute mastitis.

During a decade ago She-camels mastitis has been reported from a number of camel-rearing countries of the world such as Untied Arab Emirate (Al-Juboori *et al.*, 2013; Quandil and Quadar , 1984), Sudan (Mohamed

ELmustafa, 2014;Alamin *et al.*, 2013; Obeid, 1983), Egypt (Moustafa *et al.*, 1987; Hassanien *et al.*, 1984); India (Kapur *et al.*, 1983); Saudi Arabia (Al-Dughaym and Fadlelmula, 2015; Abdelgadir,2013; Saleh and Faye 2011; Barbour *et al.*,1995; Hafez *et al.*, 1987) , Somalia (Abdurahman *et al.*, 1991; Arush *et al.*, 1984), Ethiopia (Bekele and Molla, 2001), Kenya (Matofari *et al.*, 2003) and Iraq (AL-Tofaily and Al rodhan, 2011).

## **Aims of study**

In Iraq, few reports have been done on the camels in general and on mastitis in particular in compare to other livestock such as cattle, sheep and goat. Moreover, little attention of mastitis as a problem was paid at herd level. Al Muthanna governorate is considered as the second highest number of camels population in Iraq, however, for the authors knowledge there are no previous reports regarding she- camel mastitis in Al-Muthanna governorate. Consequently, this study intends to study the clinical and subclinical mastitis and its etiologic agents in she-camels at 3 camel herds that reared in Samawah desert / Al Muthanna governorate using SCC, CMT, in addition, to isolate and identified of the bacterial causative agents.

# Review of literatures

## **Anatomy of udder in she-camels**

In the prepuberal and nulliparous females, only the small teats are visible as the mammary tissue does not develop until the end of the first pregnancy. At the peak of lactation, udder increases in size and shows well-developed venous drainage. The udder of the camel consists of four glandular quarters, each with its own teat. The left and right halves of the udder are separated from each other by fibro elastic tissue extending from the linea alba and glandular units of the lobule, the alveoli or acini, are separated from each other by the interlobular connective tissue (Smuts and Benzuidenhout, 1987). The duct system begins with small interlobular ducts that enlarge progressively and each duct is lined by an epithelium resting on a distinct basement membrane. The duct epithelium is low, simple and secretory in the smallest interlobular duct but becomes columnar in the larger ducts (Nosier, 1974). The color of mammary gland is brown to black tinge. The anterior and posterior quarters are independent, but no visible separation between them is observed. The teats are directed cranio-ventrally, but the conformation of teats changed markedly with change in physiological state, turned noticeably round at the tips in lactating females. The circumference and diameter of teat increased from tip to base. The most striking feature observed was the presence of two-streak canals in all four teats of female camels which are longer in lactating periods (Kausar , 2001). There is a great variety in different udder and teat shapes and sizes of she-camels according to age and stage of lactation (Albrecht, 2003 and Wernery *et al.*, 2004). In lactating animals, mammary gland is characterized by major changes including increase in number of alveoli, alveolar lumen and decrease in connective tissue (Holland and Holland, 2005 and Patel *et al.*, 2007). Ultrasonographic appearance of mammary gland and teat demonstrated that teat wall could be divided into 3 layers and the base of the teat, the annular folds, appear as a hyperechoic linear structure extending into the lumen. The glands of sinuses appear as an anechoic area continuous with the teat sinus. The lining of the wall of the glands sinus appear as mixed hyper to hypoechoic areas within the hypoechoic material of the glands (Abshenas *et al.*, 2007).

## **Camel milk**

Milk is an important nutrient in human nourishment. In some communities, camels represent the most important source of this nutrient. Some projects,

for example, the one sponsored by SNV (Netherlands Development Organization) through the Resource Mobilization Center in Kenya, have demonstrated that the rational use of this animal is highly valuable for feeding poor populations (Musinga *et al.*, 2008). The milk is either consumed in the raw state (fresh), soured or used to produce yogurt or cheese. There is no need of its being boiled as much as cow or goat milk. It has a strong flavor and salty taste because camels are fond of grazing on sodium-rich herbs and shrubs. It must be drunk slowly to allow the stomach to digest it. Consequently it has an apparent effect, especially on the foreigner (person who drank it in first time); but after a short time usually gets accustomed to it, likes it very much and suffers no ill effects (AL-Ani, 2004). Also camel milk has properties that it can be kept for long periods than cow's milk when refrigerated and even with the desert heat it does not spoil shortly (Thiagarajan, 2001). Moreover, the milk composition of dromedary camel is excellent from a nutritional view point (Gran *et al.*, 1991). Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and higher concentration of vitamin C in comparison with cow milk (Barłowska *et al.*, 2011). It can be considered as a good source of minerals, vitamins and characterized by higher ratio of lactoferrin. Moreover, camel milk could meet a big part of the daily needs of humans from these nutrients because camel milk has most the essential nutrients (Al-Otaibi and El-Demerdash, 2013). The milk of camel has several beneficial characteristics, such as the absence of diabetes in populations that consume it and tolerance by patients who show intolerance to lactose. Even though camel milk does contain lactose, it is a nutrient for individuals who are allergic to cow milk. Also is much more nutritious than that of cow milk because it is low in fat and lactose contents, and higher in potassium, iron and vitamin C. Camel milk has medicinal properties and contains protective proteins, which may have a possible role for enhancing the immune defense mechanism. Its specific properties, particularly its anti-infectious action, should be used to replace other milks (Roberto *et al.*, 2013). The triglycerides, which contain a great variety of fatty acids, are accompanied with small amounts of monoacylglycerols, cholesterol, free fatty acids and phospholipids. The ability of camel milk to inhibit growth of pathogenic bacteria and its relation to whey lysozyme has been demonstrated by Barbour *et al.* (1984). The milk let-down of camels is usually stimulated by a suckling calf and is of short duration. Therefore, the calf is quickly removed and the camel is milked by milkers on both sides of the animal simultaneously. There are a number of scientific reports concerning the milk yield of camels in nomadic areas of the world (Knoess, 1976). Machine milking of camels has been carried out in Russia, Saudi Arabia, United Arab Emirates and India. The calf is still to initiate letdown, but exogenous oxytocin has also been used. Unlike cows, camels

do not milk in the udder, and any distraction at milking can stop the milk flow entirely (Al-Ani 2004). Camel dairy farming has not been properly developed. However, in certain countries such as Saudi Arabia, United Arab Emirates, Mauritania, and Kazakhstan large-scale camel dairy farms have been established. Camel's milk is one of the most valuable food resources in arid and semi-arid zones. Camel milk products such as ice cream, butter, cheese, yogurt and fermented camel milk have been produced (AL-Ani, 2004).

## **Mastitis in Camel**

Mastitis is a complex disease occurring world-wide among dairy animals, with heavy economic losses. Mammary infections results in milk compositional changes such as increase in leukocyte counts, leakage of plasma proteins into the milk and cell damage, resulting in leakage of intracellular constituents into milk, change in ion composition and decrease in milk production (Bhikane and Kawitkar, 2000 and AL-Ani, 2004). This result in reduced milk yield, degradation of milk quality and additional cost in the care and treatment of mastitis (Eyssu and Bekele, 2010). Incidence of mastitis may increase in dairy camel due to hand milking and teat malformation (Almaw and Molla, 2000). Cases of mastitis in camels have been reported from a number of camel keeping countries including Egypt (Mostafa *et al.*, 1987 and Younan and Abdurahman, 2004), Saudi Arabia (Barbour *et al.*, 1984; Saleh and Faye, 2011 and Aljumaah *et al.*, 2011), United Arab Emirates (Al-Juboori *et al.*, 2013), Iraq (AL Tofaily and Alrodhan, 2011), Jordan (Hawari and Hassawi, 2008), Morocco (Khedid *et al.*, 2003), Ethiopia (Abdel Gadir *et al.*, 2006 and Abera *et al.*, 2010), Kenya (Younan, 2002; Matofari *et al.*, 2005 and Wanjohi *et al.*, 2013), Pakistan (Ahmad *et al.*, 2012), Nigeria (Shittu *et al.*, 2012), India (Mody *et al.*, 1998), Israel (Guliye *et al.*, 2002) and from different parts of Sudan (Obied *et al.*, 1996; Amel, 2003; Suheir, 2004; Sanaa, 2005 and Alamin *et al.*, 2013). The causative agents of bovine mastitis are well defined. There is an extensive literature on bovine mastitis and to a lesser extent on ovine and caprine mastitis. In contrast, there is paucity of information about the etiological agents associated with camel mastitis. Few available studies indicate that some bacterial infections have been implicated as causes of mastitis in camels. Some of these are *Staphylococcus aureus*, *Streptococcus spp.* (Younan *et al.*, 2001; Amel, 2003; Suheir, 2004; ;Sibtain *et al.*, 2012; and Alamin *et al.*, 2013), *Micrococcus spp.* (Al-Ani and Al-Shareefi, 1997 ; Hawari and Hassawi, 2008 and Al-Juboori *et al.*, 2013), *Streptococcus agalactiae* (Younan *et al.*, 2001 ; Abera *et al.*, 2010 and Husein *et al.*, 2013), coagulase negative staphylococci (Abdurahman *et al.*, 1995), *Staphylococcus epidermides*, *Pasteurella*

*haemolytica* (Al-Ani and Al-Shareefi, 1997 and Hawari and Hassawi, 2008), *Escherichia coli* (Al-Ani and Al-Shareefi, 1997; Kalla *et al.*, 2008 and Eyassu and Bekele, 2010). and *Corynebacterium spp* (Barbour *et al.*, 1984; Abdel Gedir, 2001; Suheir, 2004 and AL-Tofaily and Alrodhan, 2011). Camel mastitis has been estimated to affect more than 25% of lactating she-camel (Saleh and Faye, 2011 and Alamin, *et al.*, 2013). It is also known to cause approximately 70% losses in milk production (Fazhani *et al.*, 2011). Mastitis can be divided into subclinical mastitis and clinical mastitis.

### **A. Subclinical mastitis**

Subclinical mastitis is very common but cannot be detected by physical examination of either the camel or udder or milk. However, there can be large numbers of somatic cells produced by the inflammation in the affected gland. In such cases the diagnosis of mastitis depends largely on the leukocyte count of milk by indirect tests such as California Mastitis Test (CMT) and Somatic Cell Count (SCC) as well as bacteriological examination (Abdurahaman *et al.*, 1996; Obeid and Bagadi, 1996 and Alkaw and Molla, 2000). The results in milk with a high somatic cell count (SCC) which is expressed as cells/ml, with subclinical mastitis can contribute a significant proportion of bulk tank (SCC). If found above 250 000 cells/ml detected that quarter of she-camel was affected with subclinical mastitis (Radostits *et al.*, 2000).

## **2. Clinical mastitis**

Clinical mastitis causes abnormalities in udder or milk and these can be detected during physical examination and systemic signs. The clinical mastitis in camel is diagnosed by palpation and examination of udder or milk, acute mastitis has been reported to occur during the first few days following parturition by alarming including anorexia, fever, general depression, swelling, severe inflammation and pain of the udder (Quandil and Oudar, 1984; Obeid and Bagadi, 1996 and Tibary and Anuassi, 2000). Chronic mastitis can be observed by presence of pus or high bacterial cell count using California Mastitis Test (CMT), atrophy of one or more quarters and presence of pustules on the surface (Barbour *et al.*, 1984, Saad and Thabet, 1993).

### **Camel mastitis in Iraq**

In mastitis in camel has been investigated by field survey in Middle Euphrates in Iraq (AL-Tofaily and Al rodhan , 2011).). A total of 402



quarters of 141 lactating she-camels were examined and many bacterial organisms were isolated. this study show that percentage of clinical mastitis was 5.22 % and 11.35% for quarters and animals respectively. 23.81% and 18.75% of quarters and animals respectively were showed acute form of mastitis, whereas 57.15% and 56.25% which identified as chronic form for quarters and animals respectively, also result showed that 19.04% samples identified as bland teats. Gram positive bacterial isolates was (76.19%) including *Staphylococcus aureus*, *Staphylococcus hycus*, *Streptococcus agalactiae* , *Micrococcus luteus*, *Arcanobacterium pyogenes*, whereas gram negative bacterial isolates was (23.8%) which included *Mannhiemia haemolytica* *Salmonilla spp* and, *Klibcilla pneumonia*. The results of study showed that varieties of ages and number of calving were not significant differences ( $\leq 0.01$ ) on clinical mastitis in Iraqi she-camels. Antimicrobial drugs against bacterial isolates showed high susceptibility to Ciprofloxacin, Doxycycline, sulphthazin/Trimethiprim, Gentamicin, and Tetracycline, others antimicrobial Chloramphenicol, and Streptomycin showed moderate sensitivity, while all bacterial isolates were found resistant to Ampicillin, Erthromycine and Trimethiprime.

### **Predisposal factors of camel mastitis**

Traditional husbandry systems and bad milking habits include tying the teats with soft bark to prevent the calf from suckling and cauterization of the udder skin by the piece of wood and cloth, which aggravates the existing lesion like wounds on teats and leaves behind scar tissue. Through these wounds the *Staphylococcus spp.* which is usually found in wounds, may invade the mammary gland tissues and contribute to the development of mastitis in camels (Younan and Abdurahman, 2004 and Alamin *et al.*, 2013). Tick infestation causes skin lesion which may facilitate bacterial entry and leaves behind permanent tissue damage especially by *Staphylococcus spp.* and *Streptococcus spp.* In a limited study in Kenya, 22% of tick bite lesions were shown to harbour *Streptococcus agalactiae* (Younan and Abdurahman, 2004). In one study in Ethiopia, 72% of udders were infested by ticks. The incidence of mastitis was higher (30%) in heavily infested udders than in noninfested udders (9%) (Almaw and Molla, 2000). Mastitis prevalence was significantly affected by tick infestations according to study reported by Abera *et al.*, (2010). Camel-pox was an important predisposing factor; causes skin lesions on teats or canal orifices. It is a contributing factor in spreading the intramammary gland infection caused by *Streptococcus agalactiae* (Younan *et al.*, 2001). Teat canal blockage with dilatation of the

gland is a common predisposal factor for camel mastitis (Younan *et al.*, 2001).

### **The important causative agents of she-camel mastitis**

#### ***Staphylococcus aureus***

Staphylococci are Gram-positive cocci, catalase positive and oxidase negative. *Staph aureus* is the most important cause of mastitis and in many cases of mastitis begins as a consequence of the penetration of pathogenic bacteria through the teat duct in to the interior of the mammary gland (Quinn *et al.*, 1994). The capacity to coagulate plasma, the principal characteristic of the *Staphylococcus aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host (Murray *et al.*, 2006), and can contaminate milk when there is an infection of the mammary gland by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking.

#### **Coagulase negative *Staphylococci***

They are non motile, non spore forming, Gram-positive, facultatively anaerobic, clustering cocci that produce catalase and glucose fermentation (Barrow and Feltham, 2003). Coagulase negative *Staphylococci* are found in skin of the external orifice of teat canal, are normal floras of the skin and considered to be opportunistic pathogens (Irlinger, 2008). The proportions of isolated from camel of these were by (Abdurahman *et al.*, 1995 and Hawari and Hassawi, 2008) especially high in subclinical mastitis and also more commonly isolated in clinical mastitis.

#### ***Streptococcus agalactiae***

These microorganisms are Gram-positive, cocci, 0.6-1.2 µm diameter, not motile, do not form spores, are catalase-negative and grow in pairs or chains, based on the presence of a polysaccharide in the cell wall. This polysaccharide is composed of galactose, N-acetylglucosamine, rhamnose and glucitol phosphate (Schuchat, 1998). *Streptococcus agalactiae* which inhabits ducts and cisterns of the gland. It causes an inflammation which blocks the ducts, leading to decreased milk production, increased somatic cell count, and eventually to involution (Harmon, 1994 and Myllys and Rautala, 1995). Among the various pathogens causing mastitis,

*Streptococcus agalactiae* is of particular importance (Meiri- Bendek *et al.*, 2002) as a causative agent in she-camel. Clinical mastitis exists in different countries; Kenya (Younan *et al.*, 2001 and Abera *et al.*, 2010) Sudan (Sanna, 2005 and Alamin *et al.*, 2013), Jordan (Hawari and Hassawi, 2008) and United Arab Emirates (Al-Juboori *et al.*, 2013) .

### ***Corynebacterium***

They are Gram-positive, catalase positive, non spore-forming, non motile, rod-shaped bacteria that are straight or slightly curved form small grayish colonies with a granular appearance, mostly translucent, but with opaque centers, convex, with continuous borders (Yassin *et al.*, 2003), with a length of 1 to 8  $\mu\text{m}$  and width of 0.3 to 0.8  $\mu\text{m}$ , which form ramified aggregations in culture. *Corynebacterium bovis* is a pathogenic veterinary bacterium that causes mastitis and pyelonephritis in cattle, and spread from cow to cow most commonly through improper milking technique. (Hirsbrunne *et al.*, 1996). In some studied on she-camel mastitis isolated *Corynebacterium bovis* as mean causative agent of mastitis (Suheir, 2004 and Alamin *et al.*, 2013) Sudan, (AL-Tofaily and Alrodhan, 2011) Iraq and (Abdel Gedir, 2001) Ethiopia.

### ***Escherichia***

*Escherichia* is Gram negative rod, non-sporing rod, often motile, catalase positive, oxidase negative, attack sugars fermentatively and aerobic and facultatively anaerobic grows (Barrow and Feltham, 2003). The proportion of *Escherichia coli* as a causative agent in she-camel clinical mastitis varies between countries, (Amel, 2003 and Sanna, 2005) Sudan, ( Kalla *et al.*, 2008) Nigeria, (Eyassu and Bekele, 2010) Ethiopia and (AL-Tofaily and Alrodhan, 2011) Iraq.

### ***Micrococcus***

*Micrococcus* is Gram-positive cocci in small or large clusters, aerobic, not motile, non-sporing, catalase positive, usually oxidase-positive and attack sugars oxidatively or not at all (Barrow and Feltham, 2003). Members of this genus have been associated with camel mastitis and it was isolated from same mastatic milk by Suheir, (2004) in Sudan Hawari and Hassawi, (2008) in Jordan and Al-Juboori *et al.*, (2013) in United Arab Emirates as important causative agent of camel mastitis.

### ***Bacillus cereus***

*Bacillus cereus* is a Gram-positive, facultatively anaerobic, spore producing, motile, rod shaped bacterium. Its spores are ellipsoidal, sub terminal and do not swell the sporangium. *Bacillus cereus* cells tend to occur in chains and the stability of these chains determines the form of the colony, which may vary from strain to strain (Logan and De Vos, 2009). The *Bacillus cereus* is a main causative agent of all types of she-camels mastitis (Hafez *et al.*, 1987 and Ramadan *et al.*, 1987), and were isolated from various countries; Sudan (Salwa, 1995 and Alamin *et al.*, 2013), Ethiopia (Eyassu and Bekele, 2010) and Kenya (Wanjohi *et al.*, 2013).

### ***Salmonella***

Gram-negative rods, motile, aerobic facultatively anaerobic, catalase positive, oxidase negative and attack sugars by fermentation with production of gas (Barrow and Feltham, 2003). In mastitic milk in one study in Iraq was found 9.52% of bacterial isolated in clinical mastitis (AL-Tofaily and Alrodhan, 2011).

### **Fungal infection**

Mycotic mastitis in camels is relatively uncommon. But some yeast was isolated from camel mastitic milk samples (Salwa, 1995; Amel, 2003 and Suheir, 2005).

### **Diagnosis of mastitis**

#### **A. Physical examination**

##### **Visual examination**

Visual check may detect the three types of clinical mastitis by examining the udder for edematous swelling, redness and visible alteration of the color and consistency of milk, watery and with clots are signs of acute mastitis. Hard atrophied, misshapen and fibrotic quarters, massive dilatation of quarter and accumulation of dried pus, exudates and hypertrophy of the teat all these are signs of chronic mastitis. Gland reveals initial, enlargement, redness and darkness end of teats or blue color of the udder are signs of gangrenous mastitis (Kelly, 1984 and AL-Tofaily and Alrodhan, 2011).

##### **Palpation**

In acute mastitis palpation of mammary gland will reveal the presence

of heat, swelling and pain in the effected quarters and increase or moderate enlargement of supramammary lymph nodes. The inflammatory reaction related to severity of mastitis and is indicated by elevated temperature, increased respiratory and pulse rates. Chronic mastitis is characterized by hypertrophy and with palpation the fibrotic regions are painless and hard with an uneven surface. The udder temperature is normal; there is an increase in size of the supramammary lymph nodes with hard content. Gangrenous mastitis is characterized by abnormal texture and there may be desquamation of the udder from the body with swelling of offensive odour. Restlessness, poor appetite, and fever are found (Kelly, 1984 and AL-Tofaily and Alrodhan, 2011).

## **Chemical examination**

### **California Mastitis Test (CMT)**

Also called Rapid Mastitis Test (RMT). It is a direct test that grossly measures the amount of DNA, primarily a function of the number of nucleated white blood cells in milk. California Mastitis Test (CMT) is based on the amount of gelling that occurs as equal amounts of milk and reagent interact; the test subjectively read after about 20 seconds. The reaction is scored visually as negative (N) no infection, trace (T) possible infections, slightly or weak positive (1), moderate or distinct positive (2) heavy or strong positive (3) (Schalm and Noorlander, 1957). It is economical, easy and rapid and can be used to detect sub-clinical camel mastitis (Sena *et al.*, 2000; Hawari and Hassawi, 2008 and Eyassu and Bekele, 2010).

### **Modified White Side Test**

The white side test is performed on glass slide onto black ground, by adding 4% sodium hydroxide solution to be mixed with the milk of each quarter in a ratio of 1:5. The she-camel is considered mastitic when it's milk become viscid and thick (separate to water and shred or flakes) (Saad and Thabet, 1993).

### **Somatic Cell Count (S.C.C)**

The somatic cell count (S.C.C.) is done according to standardized cell count methods (Packard *et al.*, 1992). An amount of 0.01 ml milk sample is spread over an area of 1 cm<sup>2</sup> on a glass slide. The smear is dried and heated slowly to prevent cracking and peeling. The smears are stained with Newman's stain for two minutes, then washed gently in water and counted.

Reading above 250,000 cell/ml is considered positive (Radostits *et al.*, 2000). The leukocytes were counted according to the following equation:

$$\text{Leukocyte count} = \frac{\text{Number of leucocytes counted} \times \text{MF}}{\text{Number of field counted}}$$

magnification factor (MF)  $\frac{40000}{3.1416 \times d^2}$  (d=diameter of microscopic lens)

# Materials and methods

## Study Area

This study was performed by visiting three camel herds in the desert of Samawah / Al Muthanna governorate 280 kilometres (174 mil) southeast of Baghdad and it is located midway between Baghdad and Basra, at the northern edge of the governorate. Al Muthanna governorate area is 51,740 km<sup>2</sup> and a dry desert climate, in summer temperatures easily exceed 40°C, while rainfall is very limited and limited to the winter months. This area is sandy with ridges, and it is desert, the camel's owners live a nomadic life, migrating from place to another looking for grassland and Water oases. The area is covered with desert plants and periodic pastures of diverse concentrations. The average High Temperatures are 15°C (January) to 42°C (July), while, the average Low Temperatures are 7°C (January) to 30°C (July) (Figure. 1).

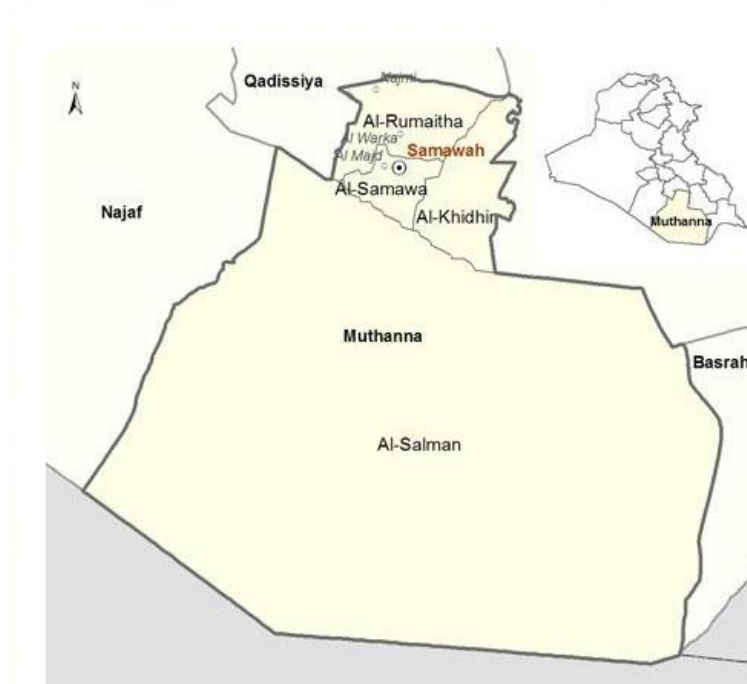


Figure.1: Shows the map of Al Muthanna governorate

## Milk Samples Collection

Thirty milk samples were collected from apparently clinical normal she-camels within 3 camelids herds within the time period between December 2016 to March 2017. These camels were grazed freely in the desert, but were also supplemented with concentrate feed that prepared as a ball. Moreover, age, lactation number, stage of lactation, pregnancy and



previous mastitis history were reported for each examined she-camels (Figure.2). The she-camel does not give the milk without stimulation factors that include feeding with a balls of concentrate (Figure.3), drinking water and allowed her calves to suckle. The milk samples were aseptically collected. The udder and the teats were washed and cleaned with 70% alcohol. The first few strips of milk from each quarter were discarded. About 100ml -200ml of milk was then collected into sterile containers. The samples were kept on ice during transportation to the laboratory and kept in the refrigerator until doing all diagnostic procedures. The quarter milk samples were subjected to bacteriological isolation and mastitis screening tests including the following:



Figure. 2: Shows one herd of camels that included in this study



Figure.3: Shows the concentrated food that given to the she-camels



## Physical examination of milk

All milk samples were examined for physical examination tests including colour, ph , consistence ( Figure 4).



Figure.4: Shows laboratory tests for milk samples

## California Mastitis Test

The test was carried out according to manufacturer's recommendation (Bori-Vet, Denmark). The test scores were as follows: negative: no thickening homogenous; trace: slight thickening that disappears in 10 seconds; 1: distinct thickening, no gel; 2: thickens immediately and begins to gel; 3: clear gel formation with surface elevation. (Figure.5)



Figure. 5: Shows the paddle of California Mastitis Test and the test

## Somatic Cell Count

The slide count was done by spreading a fine smear of a fresh milk sample on a slide. The smear was air dried and immersed in xylene for 2 minutes

to remove fat globules. Then the slide was stained with methylene blue, washed with distilled water and dried by air. The cells with blue stained nucleus were counted microscopically in 50 fields and the average number of cells per field was multiplied by the microscopic factor( Guliye *et al.*, 2002).

### **Bacteriological Cultivation**

Each specimen was cultured in duplicate onto 5% sheep blood agar, Mac Conkey's agar (Oxoid), Hayflick modified medium (for isolation of *Mycoplasma* spp.). Presumptive identification of bacterial species was done as described by (Koneman *et al.*, 2005) and confirmed by the API (bioMerieux, Inc. France).

# Results

## Results of physical examination of the milk samples

The pH of fresh camel milk varied from 6.1 to 6.5. The temperature values were ranged between 38.2 and 38.9 in all the milks. All milk samples revealed bright white colour with upper thick creamy layer.

## Prevalence of Clinical Mastitis and subclinical mastitis

No any signs of clinical mastitis were observed in all examined she-camels, so the prevalence rate was zero. Meanwhile, keratosis of the teats and udder due to severe ticks infestation were observed in 25 out of 30 she camels with percentages of 83.33% revealed (Figure.6 A& B). The subclinical mastitis was determined in 9 out of 30 with a percentage of 30% in lactating she-camels using SCC, CMT (Figure.7). The values of CMT and SCC for the specimens from apparently healthy and mastitic she-camels were as follows:

SCC	CMT
453.275	Negative
495.022	Trace
499.250	1
665.500	2
890.500	3



Figure.6: shows keratosis and ticks infestation.

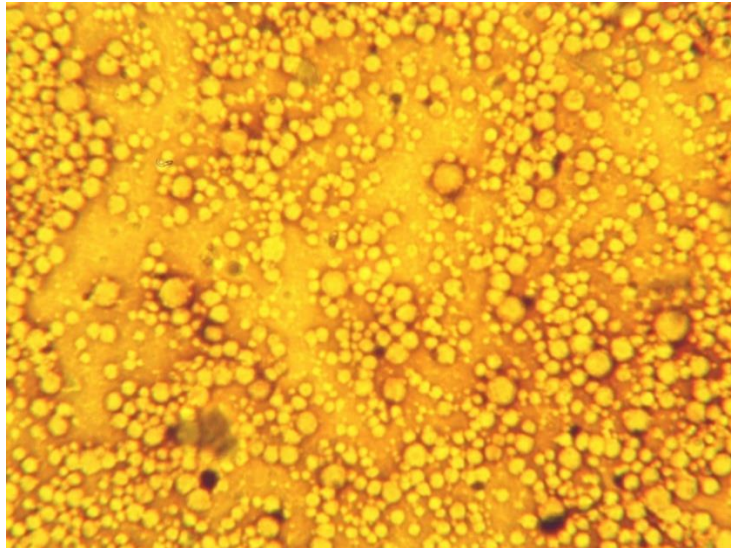


Figure.7: shows the SCC in milk

Negative or trace scores of CMT were measured as healthy and 1,2 and 3 infected. The average SCC from healthy camels ( $n = 21$ ) was determined to be 453,275 cells/ml, hence counts from 453,275 to below 499,250 cells/ml which relate to CMT score 1, were assigned to subclinical infection. From 30 milk specimens, tested by CMT and SCC, 9 specimens were positive for subclinical mastitis giving a prevalence rate of 30%.

### Microbiological Investigation

Bacteria were isolated from the 9 cases that were revealed positive results in CMT and SCC (Figure.8). The *Enterobacterium spp.*, *Staphylococcus spp.* and *Streptococcus spp.* were the most important organism isolated from the subclinical mastitis milk samples and the percentages of isolation were 55.55% (5 out of 9) , 33.33% (2 out of 9) and 11.11% (1 out of 9) respectively.



Figure. 8: Shows the growth of Enterobacteriaceae on MacConkey's Agar (MAC).

# Discussion

The results of the current study showed that camel milk revealed the colour of bright whiter colour in all samples. This results is compatible with previous results that revealed the dromedary camel milk fat contains smaller amounts of short chain fatty acids (Abu-Lehia, 1989) and a lower content of carotene. This lower carotene content could explain the whiter colour of camel milk fat (Stahl *et al.*, 2006). The current study also revealed that the pH of fresh camel milk varied from 6.1 to 6.5. This result is in agreement with previous study (Khaskheli *et al.*, 2005). The physical test of camel milk was linked to several factors such as the ingestion of some salt-tolerant plant that makes it salty and also on the food and water availability (Farah, 1993).

The diagnosis of clinical mastitis is based on a thorough physical examination; evaluation of the secretion for consistency, color, viscosity, presence of debris, and sediment and also the systematic clinical signs that appear of the infected animals. The prevalence rate of the clinical mastitis was zero in this study. And no she-camels revealed a signs of clinical mastitis. The absence of clinical mastitis may be associated with fact that she-camel is not like other lactating animals that secret and keep the milk in its mammary glands and the milk available at any time. The udder of lactating she-camel is empty and need some stimulation factors to secret the milk such as allowing her calve to suckle it. However, the field observation revealed that the milking period was very short and even the bedouin (nomadic people) believed that she-camel is a stingy and don't give milk. These observation may attribute to absence or decrease of the prevalence of the clinical mastitis in she- camels, because there is no stagnant milk, moreover, the udder is empty of milk that consider as an ideal media for bacteria growth.

CMT and SCC tests were used to diagnose subclinical among she camels in the study area. Milk specimens were obtained from 30 apparently healthy she-camels to diagnose subclinical mastitis; CMT scores of negative or trace were considered healthy and 1,2 and 3 infected. The average SCC from healthy camels was determined to be 453,275 cells/ml, hence counts up to 499.250 cells/ml that related to CMT score 1, were allocated to subclinical infection. The prevalence of subclinical infection was 30%, among randomly-selected milk samples from healthy camels, in the present study. Another study from the Saudi Arabia reported a prevalence rate of 33% based on CMT alone (Aljumaah *et al.*, 2011). However, a previous investigation suggested that CMT has about 70% sensitivity and 91% specificity in camel mastitis (Younan *et al.*, 2001). From the findings of the present study, it appears that both SCC and CMT

are sensitive in detection of subclinical mastitis. The same milk samples were tested microbiologically, where bacterial were obtained from 9 specimens which is, more or less, matching with the prevalence rate of subclinical mastitis. Still more studies are needed to correlate physical tests and microbiological tests in camel subclinical mastitis to draw solid conclusions.

## Conclusions

In conclusion, this study confirmed the correlation between SCC and CMT in diagnosis of subclinical mastitis in she-camel. This study approved the absence of clinical mastitis in she-camel due to the nature of the milk production. However, subclinical mastitis were observed in 30% of the examined animals. Early detection of subclinical mastitis and interference may aid in disease control. Various mastitis pathogens were identified from subclinical mastitis, with relatively high prevalence of *Enterobacteriaceae*. The authors recommend doing another future studies and including large number of the animals, in addition to study the natural physiological phenomena of milk production in the she- camels.

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