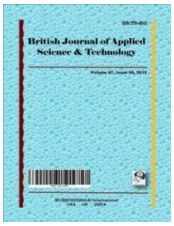

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Prevalence, Etiology and its Seasonal Prevalence of Clinical and Subclinical Camel Mastitis in Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between both authors. Author AMA designed the study, wrote the protocol, searched literature and cared for administrative affairs in samples collection. Author AF managed laboratory work, performed statistical analysis and wrote the first draft of the manuscript. Both authors read, amended and approved the final manuscript.

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ABSTRACT

A cross-sectional study to investigate prevalence, seasonal prevalence and causative agents of clinical and subclinical dromedary camel mastitis in Saudi Arabia (KSA) was conducted. The prevalence of acute mastitis was 3.6% and of chronic mastitis was 2.2%. Physical tests, California mastitis test (CMT) and somatic cell count (SCC), were done in normal and infected milk specimens to draw a borderline between clinical and subclinical mastitis; a good correlation was detected between them. The average SCC in normal camels was 478,153 cells/ml which corresponds to negative score in CMT. Based on physical tests, subclinical mastitis has a prevalence rate of 44.4%.

Many bacterial and fungal species have been isolated from mastitic cases; for a portion of them this constitutes the first report from camel mastitis. From camels' acute mastitis the most prevalent isolates were *Staphylococcus aureus* and *Streptococcus agalactiae* with fungi constituting 10.8% of all isolates. From chronic mastitis, highest rates were for Staph aureus, coagulase-negative

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Staphylococci and Corynebacterium pseudotuberculosis; fungi at 20.1%. From subclinical mastitis, Staph aureus and coagulase-negative Staphylococci were most prevalent with fungi at 12.4%. Seasonal prevalence of mastitis pathogens showed that environmental pathogens prevailed in winter and contagious pathogens in summer. From the findings of the study, the importance of mycotic mastitis is stressed. Improvement of diagnostic techniques to facilitate treatment according to etiology was highlighted. Results were discussed and conclusions drawn to improve mastitis control.

Keywords: She camels; subclinical; clinical mastitis; prevalence; etiology; Saudi Arabia.

1. INTRODUCTION

The one-humped camel (*Camelus dromedarius*) inhabits the arid and semi-arid zones of the Middle East and Africa. Its milk is extensively consumed by the nomads as fresh, raw, soured or processed into cheese [1]. In farm animals, mastitis, inflammation of mammary gland tissue, is important from the aspects of animal health and productivity, public health and economy. Camel mastitis is relatively not well studied in camel-rearing areas all over the world especially the prevalence of subclinical mastitis. Mastitis appears in two forms: clinical mastitis, where symptoms are visible and easy to diagnose, and subclinical mastitis where symptoms are invisible and require indirect means for diagnosis [2]. Reports indicate that subclinical mastitis affects animal health, reduces milk quantity and quality, impairs preservation and processing and is a public health concern for camel milk consumers [3,4]. A study was conducted on the relationship between California mastitis test (CMT) and bacterial count in the K.S.A. It has shown that a high percentage of mastitic cases were positive in CMT and there was a significant difference between the number of bacteria in CMT positive and negative cases [5]. In other studies from the Sudan, values of CMT, somatic cell counts (SCC) and bacterial isolates were compared. It was demonstrated that average values of CMT and SCC were higher in specimens from infected quarters and no bacterial isolates were recovered from a significant number of cases with high values of CMT and SCC [6]. Also it was reported that a ratio of 47.3% of 336 milk specimens tested positive in CMT and in SCC of 757 specimens, the count range was $5X10^5$ to $7.5X10^6$ cell/ml [7]. It was deduced that CMT has about 70% sensitivity and 91% specificity in camel mastitis [8]. Further, it was suggested in another study that SCC is a positive indicator of subclinical mastitis in camels more than N-acetyl-beta-D-glucoseaminidase test [9]. A recent investigation using infrared thermography technology for early detection of subclinical mastitis in camels by measuring the udder surface temperature (UST); a high correlations were obtained between UST and SCC score [10]. The prevalence of subclinical mastitis in camels in Riyadh, Saudi Arabia was reported to be 33% of tested quarter milk samples based on CMT [11]. In other studies, it was reported that overall prevalence of mastitis was 44.8% (156/348), comprising clinical (19, 5.4%) and subclinical (137, 39.4 %) [12] and 46% for mastitis prevalence with 8% for clinical and 38% for subclinical mastitis [13].

Many bacterial and fungal species have been reported as causative agents of camel mastitis. In a study in the KSA, the prevalent bacterial species were *Staphylococcus aureus*, Micrococcus spp., Streptococcus spp and *Corynebacterium* spp [5]. In another study, *Staph aureus*, Streptococcus spp and Pasteurella spp were reported [14]. [15] tested 55 milk specimens to detect subclinical mastitis and from 23 specimens, *Clostridium perfringens*, *Staph aureus* and *Escherichia coli* were identified. In a study in western Sudan, *Staph aureus,* coagulase negative Staphylococci, *Strept agalatiae* and Streptococcus spp were reported [16]. A total of 763 mastitic camels were examined and the main etiologic agents were Streptococcus spp, Staphylococcus spp, *Micrococcus* spp, *Aerobacter* spp and *E. coli* [7]. A study in Kenya reported isolation of *Staph aureus, Micrococcus* spp, *Bacillus* spp, *Strept dysgalactiae* and *E. coli* as causes of mastitis [9].

Isolation and identification of bacterial and fungal species are done using conventional methods, however, commercial identification kits are available and widely utilized. Analytical Profile Index (API, bioMerieux, Inc. France) is a miniaturized panel of biochemical tests compiled for identification of groups of closely related bacteria.

Little work has been, so far, done on camel mastitis in the Kingdom of Saudi Arabia (KSA)

despite its economic importance. The present work aimed to investigate seasonal prevalence of clinical and sub-clinical camel mastitis and its etiologic agents in the Eastern Region of the KSA.

2. MATERIALS AND METHODS

Study Area: Camel farms from the Eastern Region of the KSA. A total of 37 farms representing the region, containing 5069 she camels, traditionally-reared, were visited to investigate the prevalence of clinical mastitis.

2.1 Collection of Milk Samples

The teat tips were disinfected using pieces of cotton gauze soaked in 70% ethyl alcohol. The first stream of milk was allowed to flow out and a volume of 10–20 ml of milk was collected aseptically in labeled sterile screw-capped plastic containers. The samples were put in a cool box containing ice packs and transported to the laboratory. A total of 478 milk samples were collected from camels with clinical mastitis, as diagnosed clinically and using CMT and SCC, for a duration of 12 months, to cover all farms, in the study area. The samples were tested to identify the causative agents and compare their seasonal prevalence. As well, 263 quarter milk samples were obtained randomly from apparently healthy adult she camel for microbiological investigation and testing by CMT and SCC for the detection of subclinical mastitis.

2.2 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.

2.3 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 [9].

2.4 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.) and Sabouraud's dextrose agar (Oxoid).

Presumptive identification of bacterial species and fungi was done as described by [17] and confirmed by the API (bioMerieux, Inc. France).

3. RESULTS

3.1 Prevalence of Clinical Mastitis

A total of 5069 she camels were examined clinically for mastitis; acute clinical mastitis was diagnosed in 185 camels giving prevalence rate of 3.6% and chronic mastitis in 112 with prevalence rate of 2.2%.

Values of CMT and SCC for the specimens from apparently healthy and mastitic she camels were as follows:

CMT	SCC
Negative	478,153
Trace	500,000
1	502,300
2	714,500
3	10,871,200

3.2 Subclinical Mastitis

CMT scores of negative or trace were considered healthy and 1,2 and 3 infected. The average SCC from healthy camels (n = 146) was determined to be 478,153 cells/ml, hence counts from 478,153 to below 502,300 cells/ml which correspond to CMT score 1, were assigned to subclinical infection.

From 263 milk specimens, tested by CMT and SCC, 117 specimens were positive for subclinical mastitis giving a prevalence rate of 44.4 per 100 camels.

3.3 Microbiological Investigation

Clinical mastitis: from these cases, acute and chronic mastitis were diagnosed. Bacteria and fungi isolated from acute mastitis are shown on

Table 1 and from chronic mastitis on Table 2. All the 263 specimens tested for subclinical mastitis were cultured where pure bacterial or fungal cultures were obtained from 113 specimens with growth rate of 42.9%. The isolates are displayed on Table 3. Mycolasma spp. were not recovered from any specimen.

3.4 Seasonal Prevalence of Camel Mastitis Causative Agents

The prevalence of causative agents of mastitis during winter are shown on Table 4 and during summer on Table 5.

4. DISCUSSION

CMT and SCC tests were used to investigate subclinical and clinical mastitis among she camels in the study area. Milk specimens were obtained from 263 apparently healthy quarters 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 478,153 cells/ml, hence counts up to 502,300 cells/ml which correspond to CMT score 1, were assigned to subclinical infection. The prevalence of subclinical infection was 44.4%, among randomly-selected milk samples from healthy camels, in the present study. Another study from the KSA reported a prevalence rate of 33% based on CMT alone [11]. However, a previous investigation suggested that CMT has about 70% sensitivity and 91% specificity in camel mastitis [8]. [18] reported a positive correlation between log SCC and CMT score. As it is crucial to treat mastitis early and efficiently, methods for proper diagnosis of subclinical mastitis are always sought. An infrared thermography technique has proved feasible in early detection of camel subclinical mastitis [10], however the technique is expensive. From the findings of the present study, it appears that SCC is more sensitive in detection of subclinical mastitis than CMT.

The same milk samples were tested microbiologically, where bacterial or fungal cultures were obtained from 113 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.

Table 1. Bacterial and fungal isolates from cases of clinical acute mastitis in camels in the Eastern Region of the Kingdom of Saudi Arabia

Isolated Species	Frequency	Percentage
Staphylococcus aureus	36	19.5
Streptococcus agalactiae	17	9.2
Micrococcus spp	15	8.1
Coagulase-negative Staphylococci	14	7.6
Streptococcus dysgalactiae	14	7.6
Escherichia coli	13	7
Proteus mirabilis	11	5.9
Corynebacteriumpseudo tuberculosis	11	5.9
Mannheimia haemolytica	5	2.7
Proteus vulgaris	5	2.7
Streptococcus pyogenes	4	2.2
Arcanobacterium pyogenes	4	2.2
Streptococcus uberis	4	2.2
Streptococcus pneumoniae	3	1.6
Peptostreptococcus spp	3	1.6
Bacillus cereus	2	1.1
Bacillus spp.	2	1.1
Flavimonas oryzihabitans	2	1.1
Candida krusei	7	3.8
Cryptococcus laurentii	6	3.2
Candida tropicalis	4	2.2
Trichosporon asahii	2	1.1
Aspergillus fumigatus	1	0.5
Total	185	100.0

Table 2. Bacterial and fungal isolates from cases of clinical chronic mastitis in camels in the Eastern Region of the Kingdom of Saudi Arabia

Isolated Species	Frequency	Percentage
Staphylococcus aureus	41	21.1
Coagulase-negative Staphylococci	31	16
Streptococcus dysgalactiae	12	6.2
Corynebacterium pseudotuberculosis	24	12.4
Escherichia coli	3	1.6
Proteus mirabilis	16	8.2
Clostridium perfringens	1	0.5
Proteus vulgaris	6	3.1
Streptococcus pyogenes	1	0.5
Arcanobacterium pyogenes	2	1
Streptococcus uberis	4	2.1
Streptococcus pneumoniae	3	1.6
Peptostreptococcusspp	4	2.1
Streptomyces spp	1	0.5
Nocardia asteroides	1	0.5
Candida krusei	11	5.7
Cryptococcus laurentii	8	4.1
Candida albicans	5	2.6
Candida saki	5	2.6
Aspergillus fumigatus	6	3.1
Aspergillus niger	4	2.1
Total	194	100.0

From cases of subclinical mastitis, the most prevalent mastitis pathogen was *Staph aureus* (25.7%) followed by coagulase negative Staphylococci (21.2%) and Micrococcus spp (13.3%) together with some fungi (Table 3). Microbial recovery rate from clinical mastitis cases was 79.3%. The prevalent pathogens in acute mastitis were *Staph aureus* (19.5%), *Strep tagalactiae* (9.2%) and Micrococcus spp (8.1%) (Table 1). Recently, it has been demonstrated that *Strept agalactiae* from camel mastitis acquired tetM gene which is associated with widespread resistance to tetracycline and specific disease complexes [19]. In chronic mastitis, *Staph aureus* (21.1%), coagulase negative Staphylococci (16%) and *C. pseudo tuberculosis* (12.4%) were prevalent. Cases of subclinical mastitis do not show clear clinical signs and may unnoticed proceed to chronicity; similarity between isolates from subclinical and chronic cases, in the present study, give substance to this fact. Some of these pathogens has been reported elsewhere [20-23,5,14, 16,6,7,9],however, in the current study, many isolates has not been associated with camel mastitis in Saudi Arabia and for some like Peptostreptococcus spp, *Flavimonas oryzihabitans* and *B. cereus* this is the first report from camel mastitis. Fungi have been isolated at 10.8% in acute mastitis, 20.1% in chronic mastitis and 9.7% in subclinical mastitis. *Crypt laurentii*was isolated at a ratio of 3.2% from acute mastitis, 4.1% from chronic mastitis and 4.4% from subclinical mastitis. This is the first report of *Crypt laurentii* as a causative agent of camel mastitis and in animal diseases in general; some investigators reported association of this yeast with human diseases particularly in immune-comprimised patients [24,25]. The isolation rate of fungi from chronic mastitis (20.1%) in the present study, is rather high and almost all isolates are reported for the first time in camel chronic mastitis. Furthermore, *Candida saki* was isolated for the first time from animal diseases although it has been reported from humans with acquired immune-deficiency syndrome [26,6]. The isolated fungi could possibly be opportunistic pathogens and need further attention in camel mastitis to determine their pathogenesis. In general, overuse and misuse of antibacterial antibiotics in camel farms may predispose mycotic mastitis. It should be stressed here that the possibility exists of subclinical mycotic mastitis to progress to chronic mastitis with fibrosis and destruction of the gland while blindly trying to treat with antibacterial antibiotics.

Table 3. Bacterial and fungal isolates from cases of subclinical mastitis in camels in the Eastern Region of the Kingdom of Saudi Arabia

Isolated Species	Frequency	Percentage
Staphylococcus aureus	29	25.7
Micrococcus spp	15	13.3
Coagulase-negative Staphylococci	24	21.2
Proteus mirabilis	4	3.5
Bacillus spp.	12	10.6
Streptococcus dysgalactiae	12	10.6
Klebsiella spp	3	2.7
Candida krusei	6	5.3
Cryptococcus laurentii	5	4.4
Total	113	100.0

Table 4. Seasonal prevalence of camel mastitis causative agents during winter in the Eastern Region of the Kingdom of Saudi Arabia

Isolated Species	Frequency	Seasonal Percentage	Annual Percentage
Streptococcus dysgalatiae	11	12.6	
Micrococcus spp	11	12.6	
Escherichia coli	10	11.5	
Staphylococcus aureus	7	8	
Coagulase-negative Staphylococci	6	6.9	
Corynebacterium pseudotuberculosis	6	6.9	
Proteus mirabilis	6	6.9	
Proteus vulgaris	5	5.7	2.7
Streptococcus agalactiae	4	4.6	
Streptococcus uberis	4	4.6	2.2
Streptococcus pyogenes	3	3.4	
Streptococcus pneumoniae	3	3.4	1.6
Arcanobacterium pyogenes	2	2.3	
Peptostreptococcus spp.	2	2.3	
Bacillus spp.	2	2.3	1.1
Bacillus cereus	2	2.3	1.1
Candida krusei	1	1.1	
Cryptococcus laurentii	1	1.1	
Trichosporon asahii	1	1.1	
Total	87		

Seasonal prevalence of mastitis pathogens in the present study, showed that in winter, *Strept dysgalactia,* Micrococcus spp and *E. coli* which are described as environmental causes of mastitis prevailed. In summer, *Staph aureus* and *Strept agalactiae* which are described as contagious causes of mastitis prevailed. However, the annual prevalence rate indicated that *Staph aureus* is the most prevalent pathogen (19.5%). Camels, in the study area, are generally kept in pens during night and spend the whole daylight grazing as far as pasture is available. Due to limitation of pasture in summer, animals may be fed in the pens giving the chance for closer contacts between them which explains the prevalence of contagious causative agents. It is worth mentioning that mycotic mastitis is more prevalent in summer than winter in the present study. Effect of the hot humid weather during summer in the study area on fungal populations may be the cause but more studies on epidemiology of camel mastitis pathogens in this area are needed.

Table 5. Seasonal prevalence of camel mastitis causative agents during summer in the Eastern Region of the Kingdom of Saudi Arabia

Isolated Species	Frequency	Seasonal Percentage	Annual Percentage
Staphylococcus aureus	29	29.6	19.5
Streptococcus agalactiae	13	13.3	9.2
Coagulase-negative Staphylococci	8	8.2	7.6
Corynebacterium pseudotuberculosis	5	5.1	5.9
Proteus mirabilis	5	5.1	5.9
Mannheimia haemolytica	5	5.1	2.7
Micrococcus spp	4	4.1	8.1
Streptococcus dysgalactiae	3	3.1	7.6
Escherichia coli	3	3.1	7
Arcanobacterium pyogenes	2	2	2.2
Flavimonas oryzihabitans	2	2	1.1
Streptococcus pyogenes	1	1	2.2
Peptostreptococcus spp	1	1	1.6
Candida krusei	6	6.1	3.8
Cryptococcus laurentii	5	5.1	3.2
Candida tropicalis	4	4.1	2.2
Trichosporon asahii	1	1	1.1
Aspergillus fumigatus	1	1	0.5
Total	98		

5. CONCLUSIONS

It has been demonstrated that a correlation existed between SCC and CMT in diagnosis but SCC was more precise as it uses integers. Standardization of SCC from healthy animals would be a base in the diagnosis of subclinical mastitis.

Early detection of subclinical mastitis and interference may aid in disease control.

Various mastitis pathogens were identified from the different clinical forms, a host of which to be reported for the first time from camel mastitis, with relatively high prevalence of fungi of 10% - 20%. Identification of major mastitis pathogens such as *Staph aureus, Strept agalactiae*, *Strept dysgalactia, E. coli* and coagulase-negative Staphylococci is important to guide rational use of antimicrobial agents.

Seasonal prevalence of camel mastitis pathogens was determined in the present study to aid in disease control. Environmental mastitis pathogens prevailed in winter and contagious pathogens in summer.

Techniques for differential she -camel-side diagnosis of bacterial and mycotic mastitis are needed to decrease misuse of antibacterial antibiotics. The study highlighted importance of mycotic mastitis in she camel that needs attention in the diagnosis of clinical and subclinical camel mastitis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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