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Bacterial Causative Agents Associated with Subclinical Mastitic in Dromedary She-Camels in Southeastern Algeria

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Abstract

The present study was designed to identify the different bacterial species causing the subclinical mastitis in she-camels in Algeria, and to determine their evolution during the lactation period. One hundred and fifty three milk samples were collected from seventeen lactating she-camels from southeastern Algeria. The results showed that, 106 (69.28%) of the 153 samples examined contained bacteria, of which 84 (54.90 %) gave pure culture, 21 (13.72%) gave mixed isolations of two types of bacteria or more, and 1 (0.65%) showed contamination of culture. Furthermore, the percentage of the sub-clinical mastitis cases in the Algerian camels was not significantly different (p > 0.05) on the basis of the lactation stage. On the other hand, the Bacteriological examinations of the milk samples revealed that coagulase negative staphylococci (CNS) were the most predominant species causing the subclinical mastitis in she camels 39 (46.43%), followed by *Enterobacteriaceae* at 16 (19.05%), coagulase positive staphylococci (CPS) 15 (17.86%), and *Micrococcus* spp. at 6 (7.14%), in addition to other bacteria types. Among the CNS isolated, *Staphylococcus arlettae* (11.91%) and *Staphylococcus muscae* (9.53%) were the most dominant species. From the CPS species isolated, *Staphylococcus aureus* (7.14%) and *Staphylococcus hyicus* (7.14%) were the major species isolated, and from the *Enterobacteriaceae*, *E.coli* (10.72%) was the main species isolated. Other species. including *Bacillus cereus*. *Streptococcus* spp., *Aeromonas hydrophila*, *Achromobacter* spp. and *Flavobacterium* spp. were also isolated with low percentages.

Keywords: Algeria, Bacteria, She-camels, Subclinical mastitis.

1. Introduction

The dromedary camel (*Camelus dromedarius*) is the most important livestock species in the desert and semi-desert areas of Northern and Eastern Africa as well as in the deserts of the Arabian Peninsula (Al-Juboori *et al.*, 2013). On the whole, camels are considered as the main source of both milk and meat production in these areas (Lyer *et al.*, 2014).

The she camel milk, similar to that of other dairy animals, contains all the essential nutrients and is regarded a perfect nutritious drink (Tuteja *et al.*, 2013). Furthermore, it contains a high proportion of antibacterial substances and higher concentrations of vitamin C in comparison with the cow milk (Barlowska *et al.*, 2011). People in arid, semi-arid and desert areas consume raw camel milk as one of the main components of their diet (Siboukeur, 2007). This poses a health risk to humans since the milk is a very nutritious medium; readily supporting the growth of microorganisms originating from

environmental contaminants (Kotb *et al.*, 2010), or as a result of clinical and subclinical mastitis (Wanjohi, 2014).

Mastitis is a complex disease which affects all dairy animals without discrimination. It causes great economic losses if not detected and treated promptly (Lyer *et al.*, 2014). Mastitis can be defined as an inflammation of the parenchymal tissue of the mammary gland. Regardless of cause, it is characterized by a range of physical and chemical changes in the milk in addition to pathological changes in the glandular tissue which include swelling, heat, pain, and edema of mammary gland. The most important changes in the milk include discoloration, presence of clots and presence of a large number of Leukocytes (Radostits *et al.*, 2007).

Moreover, camel mastitis has been estimated to affect more than 40 % of the lactating she-camels (Ahmad *et al.*, 2011; Regassa *et al.*, 2013). It was also known to cause approximately a 70% loss in milk production (Fazlani *et al.*, 2011). The sub-clinical mastitis in she camels is considered as the most prevalent type (Ahmad *et al.*, 2011; Alamin *et al.*, 2013; Husein *et al.*, 2013). It refers to the existence of inflammation with an absence of gross

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inflammatory changes in the udder, making it difficult to be diagnosed early. It can be detected only through laboratory testing (Abdel Gadir, 2014). Some reports have indicated that the sub-clinical infection in the udder of dromedary causes lower milk production, and changes in the milk properties which impair the processing and preservation of the milk (Saleh and Faye, 2011). On these accounts, the comprehension of its pathogenesis and the early diagnosis are of vital importance in the treatment of mastitis (Khan *et al.*, 2013).

Bacterial infections are the primary causes of mastitis in domestic animals (Seifu and Tafesse, 2010). For this reason, many different bacteria have been isolated from mastitic mammary glands of she-camels. The major pathogens of mastitis in she-camels are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Actinomyces pyogenes*, *Escherichia coli*, *Micrococcus* spp. and *Corynebacterium bovis* (Abdel Gadir, 2014), in addition to, *Streptococcus dysgalactiae* (Husein *et al.*, 2013; Regassa *et al.*, 2013), coagulase negative staphylococci (Abdurahman, 2006; Husein *et al.*, 2013; Wanjohi *et al.*, 2013), *Pasteurella* spp., and *Pseudomonas aeruginosa* (Al-Juboori *et al.*, 2013).

According to a review study, during the last decades, cases of mastitis in dromedary camels have been reported from many of the camel-rearing counties of Africa and Asia, such as Kenya, Somalia, Sudan, Egypt, Saudi Arabia, Iraq and UAE (Abdel Gadir, 2014). There is slight literature on bovine mastitis, and to a lesser extent on ovine and caprine mastitis in Algeria. However, there is no published data about mastitis in she camels. Accordingly, the present study was undertaken to identify the most significant species of bacteria involved in causing subclinical mastitis in she-camels, and to evaluate the evolution of this disease throughout the lactation period.

2. Materials And Methods

2.1. Study Area

The present study was conducted at Bir Naam, South East of Algeria. This region is characterized by an arid climate with an average summer temperature of 42 °C, and low monthly precipitation of 10.72 mm. The rainy period extends from November to January with a 23.8 mm maximum in January, and pastures in this region are considered arid for the rest of the year. The vegetation commonly consists of steppe plants such as *Stipa tenacissima* and *Ampelodesmos tenax*.

2.2. Management of Camels

This study was conducted from November, 2014 to September, 2015. Seventeen multiparous and healthy lactating she-camels kept under grazing and supplementary farming systems were randomly selected. The she-camels were kept grazing in open areas surrounding the farm from the morning times until mid-day then they were taken inside the farm for milking and for supplemental feeding. They were given supplemental barley concentrate and dry hay straw. The animals were provided with water regularly. The calving occurs mostly during the winter season, starting as early as November. The milking was manual. A good number of the she-camels were infested with the ecto-parasite (ticks).

2.3. Sampling Procedure

The milk samples were collected during three different stages of lactation (early, mid and late respectively). Three samples were taken from each animal at each lactation stage. A total of 153 milk samples were collected for this study. Before the collection of the samples, udders of the she-camels were examined visually, and by palpating for the presence of any lesions, and for redness, pain, heat, and swelling. After that, the camels were allowed for udder preparation by washing with water and by the disinfection of the teats with alcohol 70 °C. The raw camel-milk samples were collected in the early morning in sterilized bottles after eliminating the first streams. The milk samples were labeled, stored in an ice box, and were taken immediately within 2-4 hours after collection to the laboratory for the Bacteriological analysis.

2.4. Bacteriological Analysis

All milk samples were randomly selected, and used for Bacteriological analysis for the detection of specific bacteria causing the sub-clinical mastitis. A loopful of each milk sample was streaked on defibrinated sheep (7%) blood agar, nutrient agar, BCP (Bromcresol Purple Lactose) agar, and Chapman agar. Plates were incubated at 37°C for 24-48 hours. The grown colonies were subjected to the following tests as recommended by the National Mastitis Council (NMC, 1987): the presumptive identification of the isolates based on the colony morphologic features, Gram stain reaction, hemolytic characteristics, catalase test and biochemical classic tests. Based on the Gram stain technique, there was pure Grampositive cocci, pure Gram-negative, and mixed isolates. Staphylococci and Micrococci were identified based on their growth characteristics on triple sugar iron agar, mannitol salt agar, nitrate reductase test, urease test, coagulase and catalase tests (Forbes et al., 2002; Quinn et al., 2011). Isolates that were tentatively identified as streptococci were evaluated according to growth characteristics on sheep blood agar, catalase production and sugar fermentation tests. Gram-negative isolates were sub cultured on BCP agar and were further tested using triple sugar iron agar, testing motility, the IMViC test (Indole, Methylred, Voges-Proskauer, and citrate utilization tests) and the urease test (Forbes et al., 2002; Quinn et al., 2011). Samples with a growth of 5 or more identical colonies (Contreras et al., 1997; Pradieé et al., 2012) were considered positive for subclinical mastitis. The growth of two or more morphological types (> 5 CFU per type) was considered as contamination, and the result was excluded from the analysis (Gonzalo et al., 2002; Pradieé et al., 2012).

2.5. Statistical Analysis

Data were recorded in Microsoft Excel, 2007 spread sheets for statistical analysis. Descriptive statistics were used to summarize the data and calculate the sample statistics and the various proportions. Additionally, the effect of the stage of lactation on the occurrence of subclinical mastitis was analyzed by Cochran Q Test using SPSS (version 16). Probabilities of p<0.05 were considered significant.

3. Results

The nature of the milk samples recorded in the present study is given in Table 1. Out of the 106 culture-positive milk samples examined, 84 (54.90%), 21 (13.73%) and 1 (0.65%), were determined having pure, mixed bacteria with two species or more, or contaminated cultures, respectively. It is clear from the present investigation, that pure infection was common as compared to mixed or contaminated infections (Table 1). On the other hand 47 out of 153 examined milk samples (30.72%) were bacteriologically negative cultures.

Table 1. The bacterial nature of milk samples of the she-camels.

Nature of isolates		Number	Percentage %
Culture-positive sar	nples	106	69.28
Nature of samples	Pure samples	84	54.90
	Mixed samples	21	13.73
	Contamination	1	0.65
Culture-negative Sa	imples	47	30.72
Total		153	100

The data available on the relative frequency of different types of organisms encountered in the udder infection (Table 2) revealed that coagulase negative staphylococci (CNS) were the most important organisms involved in the causes of subclinical mastitis in she-camels (46.43%). *Enterobacteriaceae* came next in significance at (19.05%), followed by coagulase positive staphylococci (CPS) at (17.86%). The percentage of *Micrococcus* spp. was (7.14%), and other bacteria types were low (9.52%) (Table 2).

Table 2. The number and percentage of the predominance of different bacteria in subclinical mastitis of she-camels.

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Isolates	Number	Percentage %
Coagulase negative staphylococci (CNS)	39	46.43
Enterobacteriaceae	16	19.05
Coagulase positive staphylococci (CPS)	15	17.86
Micrococcus spp.	6	7.14
Other bacteria	8	9.52
Total	84	100

As indicated in Table 3, the percentage of subclinical mastitis in this study was not affected significantly by the lactation stage (p>0.05).

Table 3. The percentage of subclinical mastitis in she-camels based on the stage of lactation.

based on the stage of	iactation.			
Lactation stage (months)	0-2	3-5	6-9	Total
Number of samples	51	51	51	153
Number and percentage of positive samples	29 (56.86 %)	24 (47.06%)	31 (60.78%)	84 (54.90%)
Effect of lactation stage (P-value)	NS	NS	NS	

^{*} p < 0.05, ** p < 0.01, NS: no significant p> 0.05.

Table 4 shows the identification and differentiation of bacterial species causing the subclinical mastitis in shecamels in the current study. The most common isolated CNS species were *S. arlettae* (11.91%), *S. muscae* (9.53%), *S. epidermidis* (5.95%). *S. saccharolvticus* (5.95%), *S. cohnii* (4.77%), *S. succinus* (3.57%), *S. saprophyticus* (2.38%), *S. auricularis* (1.19%) and *S.*

capitis (1.19%), respectively. Although, from the CPS species, *S. aureus* (7.14%) and *S. hyicus* (7.14%) were the most dominant causes of subclinical mastitis in she-camels followed by *S. intermedius* (3.57%), respectively. However, from *Enterobacteriaceae*, the *Escherichia coli* (10.72%) was the most dominant species, followed by *Pseudomonas aeruginosa* (2.38%), *Providencia* spp. (2.38%), *Morganella morganii* (1.19%), *Proteus mirabilis* (1.19%) and *Serratia* spp. (1.19%). Nevertheless other bacteria were isolated such us: *Micrococcus* spp. (7.14%), *Bacillus cereus* (3.57%), *Streptococcus* spp. (2.38%), *Aeromonas hydrophila* (1.19%), *Achromobacter* spp. (1.19%) and *Flavobacterium* spp. (1.19%).

Table 4. The percentage of bacterial species in subclinical mastitis of she-camels.

Gram stain result		Bacteria Species	Number	Percentage %
	Coagulase negative staphylococci (CNS)	S.arlettae	10	11.91
Gram		S.muscae	8	9.53
positive		S.epidermidis	5	5.95
		S.saccharolyticus	5	5.95
		S.cohnii	4	4.77
		S.succinus	3	3.57
		S.saprophyricus	2	2.38
		S.auricularis	1	1.19
		S.capitis	1	1.19
	Coagulase positive staphylococci (CPS)	S.aureus	6	7.14
		S.hyicus	6	7.14
	S.intermedius	3	3.57	
	Micrococcus spp.		6	7.14
	Streptococcus spp.		2	2.38
Gram negative	Enterobacteriaceae	Escherichia coli	9	10.72

	Pseudomonas aeruginosa	2	2.38
	Providencia spp.	2	2.38
	Morganella morganii	1	1.19
	Proteus mirabilis	1	1.19
	Serratia spp.	1	1.19
Other bacteria	Bacillus cereus	3	3.57
	Aeromonas hydrophila	1	1.19
	Achromobacter spp.	1	1.19
	Flavobacterium spp.	1	1.19
	Total	84	100

4. Discussion

In this study, samples containing a single bacterial organism were the most common (54.90%), than those containing two bacterial species or more (13.73%). This finding was consistent with the results of Abdurahman (2006) and Regassa *et al.* (2013), who reported that

compared to mixed infections, pure infections were more common in the milk samples of the she-camels infected with subclinical mastitis. On the other hand, Younan *et al.* (2001) reported that infections in the udder of the lactating camels are quite widespread. Generally speaking, bacteria in the milk can occur through colonization of the teat canal or an infected udder (clinical or subclinical mastitis), or as contaminants (Younan, 2004). Moreover, the current study revealed the presence of tick infestation on udders which causes skin and teat lesions. Furthermore, these lesions facilitate bacterial entry and cause permanent tissue damage (Megersa, 2010).

The results of the present study indicated that the proportion of sub-clinical mastitis cases in camels was not significantly different with the variation of the lactation stage. This result was in agreement with Ali et al. (2016). However, Ahmad et al. (2011); Aljumaah et al. (2011); Husein et al. (2013) and Regassa et al. (2013) stated that the percentage of mastitis in the early stage of lactation was significantly higher. On the other hand, Suheir et al. (2005) declared that the last stage of lactation was found to be associated with a high rate of subclinical mastitis. This variation could be attributed to other factors which were important for predisposing mastitis in she-camels such as the hygienic milking process (Ahmad et al., 2011), the use of anti-suckling devices to prevent suckling by the camel's calves, tick bites on the udder, deformities of the udder tissue due to the thorny bushes in the pastoral areas, and camel pox (Abdel-Gadir, 2014), in addition, to age (Ahmad et al., 2011), breed, parity number (Abdurhmann, 2006; Ahmad et al., 2011; Aljumaah et al., 2011) and the production system (Ahmad et al., 2011).

Among the bacterial isolates, CNS were identified in this study as the predominant organisms causing subclinical mastitis in she-camels. This finding agreed with the reports of Abdurahman (2006) and Husein et al. (2013). However, Wanjohi et al. (2013) declared that the most isolated bacterium from mastitic camel's milk in Ethiopia was Klebsiella/Enterobacter followed by CNS, respectively. Other reports stated that the CPS bacteria were the most dominant causes of mastitis in she-camels (Ahmad et al., 2011; Alamin et al., 2013; Al-Juboori et al., 2013; Regassa et al., 2013). CNS bacteria are known as the facultative or minor pathogens isolated from subclinical mastitis (Abdel-Gadir et al., 2005). The frequent occurrence of CNS was most probably due to the contamination of the samples through the teat canal or teat skin. Moreover, CNS were mainly present in air, soil and water of camels (Kotb et al., 2010) which served as a source of contamination, and were associated with subclinical mastitis in camels' udders.

The current study suggests that the Enterobacteriaceae bacteria were the second cause of subclinical mastitis in she-camels. This finding agreed with that reported by Al-Juboori et al. (2013) in UAE. In contrast to the results of this study, many authors reported that Streptococcus spp. bacteria were the second most common cause of mastitis in camel herds (Abdurahman, 2006; Seifu and Tafesse, 2010; Ahmad et al., 2011; Husein et al., 2013; Regassa et al., 2013). However, Wanjohi et reported that the Enterobacteriaceae (Klebsiella/Enterobacter and E.coli species) were the most predominant causes of she-camel mastitis in a

study conducted in Ethiopia. The contaminated environment of breeding was the main source of *Enterobacteriaceae* (Kotb *et al.*, 2010), which mostly predisposed the udders towards bacterial infections.

The percentage of CPS noted in our study was in agreement with the finding of Suheir *et al.* (2005), but it was lower than that reported by Ahmed *et al.* (2011); Alamin *et al.* (2013) and Wanjohi *et al.* (2013) in milk samples collected from Gharissa districts in Ethiopia. However, the obtained percentage of CPS was higher than that (14.3%) recorded by Wanjohi *et al.* (2013) in milk samples from Wajir districts. The frequency of *Staphylococcus* varied according to different studies, but there is practically no recent or even previous publication on the Bacteriological pathogens in mastitis affecting shecamels where staphylococci are not stated.

The present study also identified a low proportion of Micrococcus spp. which was in consistent with the findings of other studies (Saleh and Faye, 2011; Al-Juboori et al., 2013), even though Regassa et al. (2013) found lower percentage. In contrast to our results, Hawari and Hassawi (2008) recorded that Micrococcus spp. bacteria were the predominant mastitis-causing organisms in she-camels. Similarly, Suheir et al. (2005) stated that these organisms are an important causative of the mastitis among camels. This bacterium was mainly frequent in the camel environment, particularly, in soil, air and water (Kotb et al., 2010), which subjected the milk to the microbial contamination existing in the surroundings elevating the contamination. The low frequency of Micrococcus spp. observed in this study could be attributed to the existence of adequate hygiene during the milking and in the management system.

The isolated CNS species in positive samples were S. arlettae, S. muscae, S. epidermidis, S. saccharolyticus, S. cohnii, S. succinus, S. saprophyticus, S. auricularis and S. capitis. However, Alamin et al. (2013) recorded that the commonly isolated CNS species in persistent subclinical mastitis in camels were S. epidermidis, S. simulans, S. haemolyticus, S. kloosii,, S. lentus, S. delphini, S. saprophyticus, S. lugdunensis, S. sacchrolyticus, S. carnosus and S. chromogenes. In this study, S. arlettae and S. muscae followed by S. epidermidis and S. saccharolyticus were the most prevalent causative microorganisms involved in she-camel mastitis, Other authors reported that S. epidermidis was the most frequent CNS isolated from the camel milk (Abdel Gadir et al., 2005; Al-Juboori et al., 2013).

Among the isolated CPS, *S. aureus* and *S. hyicus* were the most predominant species followed by *S. intermedius*. This result was in agreement with Woubit *et al.* (2001), who recorded that *S. aureus* and *S. hyicus* were predominant subclinical mastitis-causing organisms in camels in Ethiopia. However, Alamin *et al.* (2013) reported that *S. aureus* was the main organism involved in causing subclinical mastitis in camels and *S. intermiduis* came next in significance, followed by *S. hyicus*. Other reports suggested that *S. aureus* was the most isolated CPS species (Seifu and Tafesse, 2010; Saleh and Faye, 2011; Al-Juboori *et al.*, 2013; Husein *et al.*, 2013). This is probably related to the fact that *S. aureus* is well adapted to survive in the udder, and usually establishes a mild subclinical infection of a long duration, from which it shed

in milk, facilitating the transmission to healthy animals, mainly during the milking procedure (Radostits *et al.*, 2000).

The relative number of *Streptococcus* spp. isolated in this study, was very similar to that reported by Alamin *et al.* (2013). However, it was much lower than previous studies (Seifu and Tafesse, 2010; Saleh and Faye, 2011; Al-Juboori *et al.*, 2013, Husein *et al.*, 2013). The low proportion of *Streptococcus* spp. could be explained by the possible premedication of the animals with antibiotics, especially that it is known that mastitis caused by *Streptococcus* spp. is susceptible to eradication via the use of antibiotics (Hawari and Hassawi, 2008; Alqurashi *et al.*, 2013).

The percentage of E. coli recorded in this study is equal to the earlier findings of Ahmad et al. (2011) and Saleh and Faye (2011), but it was much lower than that reported by Wanjohi et al. (2013). The low rate of E. coli isolates might be partially associated with effective udder washing and drying, post milking teat dipping, and keeping washing towels clean. Because E. coli is a common intestinal bacterium, the isolation of this bacterium was taken as indicator of fecal contamination that is contamination of one of the milking hygiene conditions. This is significant because the feces may have contained pathogenic organisms (Wanjohi, 2014). But, camel feces are dry, and do not normally contaminate the udder skin (Eberlein, 2007). However, its presence in milk cannot be avoided completely, but can be rather minimized by good management and hygienic milking practices.

The present study also identified a low rate of Pseudomonas aeruginosa, which was in agreement with that reported by Al-Juboori et al. (2013). Other bacterial species were isolated including Providencia spp., Serratia spp., Proteus mirabilis, Morganella morganii and Flavobacterium spp., but with very low percentage originating possibly from the camel environment. According to Kotb et al. (2010) Providencia, Serratia and Proteus resulted from the contamination of the water and the soil. Moreover, Younan (2004) declared that under pastoral production conditions, environmental contamination was likely to play a bigger role in contaminating the raw camel milk than the initial bacterial contamination of the camel milk. On the other hand, the camel milk has a high antimicrobial activity as compared to that of other animal species, and it's able to inhibit Gram-positive and Gram-negative pathogens. But the natural antimicrobial factors can only provide a limited protection against specific pathogens and for a short period of time (Benkerroum et al., 2003).

A low proportion of *Bacillus cereus* was recorded through the present study which complied with the results of most investigations stating that the *B. cereus* species was not a pathogen of importance in camel milk (Ahmad *et al.*, 2011; Alamin *et al.*, 2013). The high percentage of *B. cereus* reported by Wanjohi *et al.* (2013) could be due to poor management and unhygienic milking practices.

On the whole, the findings of the present study were in accordance with the observations of several previous studies, with only minor variations, possibly attributable to different geographical climates, the management system, and individual variations in susceptibility. However, the failure of some pathogen to grow in the current study such

as *Corynebacterium* spp., *Klebsiella* spp., and other mastitis pathogens could be explained by the possible premedication of the infected animals with antibiotics.

5. Conclusion

In conclusion, the results of the current study contribute to the overall knowledge about the main species causing subclinical mastitis in camels in Algeria. It is concluded from this work that pure infection was common in camels as compared to mixed infections and that CNS were the dominant mastitis isolated pathogens, followed by Enterobacteriaceae. Furthermore, S. arlettae, E. coli and S. muscae were the main frequent bacterial isolates from the camels infected with subclinical mastitis in the study area. On the other hand, the present study confirmed that the percentage of sub-clinical mastitis cases in Algerian camels was not significantly different with the difference of the lactation stage. Moreover, adequate hygienic conditions in the environment, identification of subclinical infected glands, clinical treatment of the infected shecamels, dry-period therapy, and performing a systematic regular ecto-parasite control of the livestock are all required in order to minimize the occurrence of subclinical mastitis in the study area and. therefore, reduce the adverse effects of mastitis on the production and quality of camel milk.

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