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A study on prevalence of bacteriological mastitis in dromedary camels (*Camelusdromedarius*) and its Antibiogram Profile

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Abstract: The prevalence of the clinical and sub clinical mastitis in *Camelus dromedaries* (n=488) was determined along with their causes and risk factors involved in around coastal mangroves areas of upper Sindh. Eighty-two households were selected on the basis of holding milking camel. The milk samples collected aseptically were 1190 from clinical and 378 from sub clinical out of total 1568 samples. To assess the prevalence all these samples were screened for California Mastitis Test (CMT), Surf Test and White Side Test and bacterial culture. Out of 1568 cultured quarter milk samples, 138 found positive for clinical 11.59% (138/1190) and 22.75% (86/378)for subclinical mastitis. At animal (camel) level the prevalence of clinical 50.61% (165/220) and subclinical mastitis was 33.95% (55/220camels). It was concluded that, an overall infection rate is higher in the right fore quarter (36%) as compare to left fore quarter (27%), Similarly the higher infection rate is found in right hind quarter (20%) then left hind quarter (18%) for clinical and sub clinical mastitis.

Staphylococcus aureus gram-positive bacterium was observed predominant with a prevalence of 29.26% (48/164) and Staphylococcus epidermidis 23.78% (39/164) followed by gram-negative Escherichia coli with prevalence of 20.12% (33/164). Streptococcus agalactiae and Bacillus spp. were the fourth and fifth predominant isolates with prevalence of 13.41% and 8.55% (22/163 & 14/164) respectively. Corynebacterium was the least isolates with 4.88% prevalence. An overall 30.2% (116/384) prevalence of camel mastitis was recorded with clinical mastitis 25.3% (97) and sub-clinical mastitis 4.9% (19/384). The overall prevalence was 25.8% (397/1536) in all quarters of buffaloes. The isolated organisms were analyzed for antibiogram assay using 12 commonly used antibiotics and enrofloxacin, pentamicin, and kanamycin were found most effective antimicrobials amongst all tested drugs. These results exhibited a high prevalence of mastitis in dromedary camels with significant antimicrobial resistance of bacterial pathogens. Therefore, proper prevention and control strategies are warranted in the area to minimize the economic losses of camel farmers and to avoid public health hazards.

Keywords: Camel, Prevalence, Mastitis, Bacteriology, Antibiogram

1. <u>INTRODUCTION</u>

There are two types of camels in the world: the one-humped camels (*Camelusdromedarius*) and bactrian or two - humped camels (*Camelusbactrianus*). The bactrian camel is mainly found in the regions of the European - Russian continent. The one humped camel is the most populous animal in the subtropical as well as tropical regions of Asia and Africa. Despite of living in tough milieus of arid and semi-arid areas, the dromedary camel may produce cherished quantity of milk (Younan, 2004).

In rural areas as well as pastoralists always consume camel milk fresh (without heat treatment) or with slight heat treatment. Camels are usually raised particularly during the summer season in natural harsh desert habitat and subjected to severe stress

environments which usually cause the onset of many infectious and non-infectious diseases (Agab, 1993).

Mastitis is the most devastating economical ailment of dairy animals. This disease is prevalent in dairy herds and associated with a significant decrease in milk yield. This condition also induces the significant change in milk composition with considerable rise in white blood cell counts. It also results in the damage of blood components that cause the break away from plasma proteins into the milk and influences ion composition of milk (Korhonen and Kaartinen, 1995). On the basis of disease stage, mastitis can be divided into the two classes i.e., clinical and subclinical mastitis. Subclinical mastitis requires early diagnosis (Matofari *et al.*, 2003). Camel mastitis is economically and from public health point of view is very important because of its multiple

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harmful effects (Younan, 2004; Akweya *et al.*, 2010; Njage *et al.*, 2010).

Mastitis also cause partial or whole damage of udder tissues and declines the production length of animal. The camel mastitis is caused by many types of bacterial pathogens including the coliform bacteria (e.g., Enterobacter, Klebsiella and E. coli), Staphylococci, Streptococci, Mycoplasma, Corynebacteria, Leptospira, Yersinia, Pasteurella, Serratia, Pseudomonas, Mycobacteria and other non-bacterial organisms like viruses, yeast, mold and other dimorphic fungi. There is an in adequate information available on etiology, prevalence, and antibiotic sensitivity of pathogen causing mastitis in camels in coastal mangroves areas of Thatta, Badin and Karachi districts of Sindh. Present study was, therefore, carried out to determine the prevalence of mastitis around the coastal mangroves areas of Sindh, Pakistan. This study further investigated the bacterial organisms responsible for camel mastitis along with their antibiogram pattern.

2. <u>MATERIALS AND METHODS</u> Study plan and area

A cross-sectional study was performed during a period of 6 months (July 2017 to December 2017). A single visit, multiple-subject survey (ILCA, 1990) was adopted to determine the incidence of clinical and sub clinical mastitis in camels. Regarding the sample procedure, area around Thatta, Badin and Karachi districts of Sindh were selected based on current camel population and accessibility. Samples were collected using purposive sampling technique as described by (Petrie and Watson, 1999). There was no any defined farming method, camels reared by pastoralists practicing sedentary and semi-nomadic production system. It was quite difficult to use random sampling methods. The following measures were adopted for choice of animals: (1) similar nutritional status, (2) unlike management circumstances, (3) inclination of the pastoralists to add in the study, and (4) easy approach of the location, so that collected samples could be easily conveyed to the laboratory for investigation.

Collection and investigation of milk samples

Before collection of samples, the camel owners were convinced about the objectives and purpose of the study. Physical examination of all the quarters were done of sampling camels.20 ml of milk sample from each quarter from 488 lactating camels were aseptically obtained in sterile bottles, after discarding the first few strips. Samples were kept directly into an icebox (4-8°C) and moved to Diagnostic Laboratory at the Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture

University, Tandojam for bacterial culture. During milk sample collection CMT was performed to detect subclinical mastitis (Schneider and Japer 1964 and Radostits *et al.*, 2000).

Bacteriological Isolations

To detect major bacterial pathogens related to mastitis, culturing and bacteriological examination was done by standard techniques (Pirzada *et al.*, 2016). The identification of isolates was carried out using standard biochemical and sugar fermentation characteristics (Habib *et al.*, 2015).

In vitro antibiogram of bacterial isolates

Susceptibility of all the bacterial isolates were analyzed in vitro to generally used antibiotics of veterinary practice. These were: Gentamicin, Enrofloxacin, Norfloxacin, Kanamycin, Sulphamethoxazole + Trimethoprim, Oxytetracycline, Amoxicillin, Penicillin, Colistin, Neomycin, Streptomycin and Chloramphenicol. Clinical and Laboratory Standard Institute (CLSI, 2016) recommended disk-diffusion assay was used for the antibiogram study (Khan et al., 2016).

Data analysis

The statistical observations of collected data were entered in SPSS.20. Descriptive statistics and Chisquare test were done to find out the relationship of risk factors with percent prevalence of mastitis. A*P*-value of <0.05 was considered significant.

3. <u>RESULTS</u>

Prevalence of mastitis

The results regarding prevalence of mastitis in 488 lactating camels (1568 quarters) were presented in Table 1. Out of 326 camels (1190 quarters) observed for the existence of clinical mastitis, 165 camels (138 quarters) were found to have clinical mastitis, the incidence being 50.61% (11.59% on quarter basis). A total of 162 camels were examined for estimating the prevalence of subclinical mastitis, 55 (33.95%) camels were found to have subclinical mastitis; these animals were carrying mastitis pathogens in their udder secretion. Subclinical infection of mastitis was detected in 86 (22.75%) of the 378 quarters examined. All the 488 camels examined during present study exhibited a 45.08% prevalence of mastitis on animal basis, while 14.28% was observed on quarter basis. Additionally it was observed that hind quarters were affected with clinical and subclinical infections (39.89% and 34.78%, respectively) more frequently than fore quarters (60.87 % and 65.12%, respectively) (Table 2).

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Type of infection	No. of camels examined	No. of quarters Positive No. Prevalen examined		Positive No.		ce rate (%)	
			Animals	Quarters	On animal basis	On quarter basis	
Clinical	326	1190	165	138	50.61	11.59	
Sub clinical	162	378	55	86	33.95	22.75	
Total	488	1568	220	224	45.08	14.28	

Table 1: Prevalence of clinical and subclinical mastitis in dromedary camels.

Table 2: Distribution of clinical and subclinical mastitis in dromedary camels according to the position of quarters.

Type of	No. of quarters	Distribution				
infection	infected	Right fore quarter	Left fore quarter	Right hind quarter	Left hind quarter	
Clinical	138	48 (34.78%)	36 (26.09%)	26 (18.84%)	28 (21.05%)	
Sub clinical	86	32 (37.21%)	24 (27.91%)	18 (20.93%)	12 (13.95%)	
Total	224	80 (35.71%)	60 (26.78%)	44 (19.64%)	40 (17.86%)	

Bacterial Isolation Analysis

(Table 3) depicts the isolated bacteria from 224 quarter samples. A total of 164 bacteria of 7 different microbial species were isolated. *Staphylococcus aureus* was the most prevalent pathogen 29.26% (48/164), of all isolates, followed by *Staphylococcus epidermidis* 23.78% (39/164), *Escherichia coli* 20.12% (33/164), *Streptococcus spp.* 13.41% (22/164), *Bacillusspp.* 8.55% (14/164) and *Corynebacterium* 4.88% (8/164).

Table 3. Bacterial isolation rates and their frequency percentage

Micro-organism	Total number of Isolates (n)	Frequency (%)
Staphylococcus aureus	48	29.26
Staphylococcus epidermidis	39	23.78
Escherichia coli	33	20.12
Streptococcus agalactiae	22	13.41
Bacillus spp.	14	08.55
Corynbacterium spp.	08	04.88
Total	164	100.00

Sensitivity of mastitis pathogens

During this study diverse isolates has been recovered from clinically and subclinically mastitis quarters milk samples of camels against in vitro antimicrobial susæptibility profiles (antibiogram), summarized in Table 4. In terms of percent susceptibility, gentamicin was the most effective in vitro antibiotics (84.63%) isolates being sensitive to this antibiotic, followed by enrofloxacin (72.79%), norfloxacin (70.16%),kanamycin (68.84%),Sulphamethoxazole Trimethoprim +(67.53%),Oxytetracycline (65.0%), Amoxicillin (60.16%),Penicillin (59.64%), Colistin (57.10%), Neomycin (54.37%), Streptomycin (38.16%) and Chloramphenicol (28.85%) respectively.

Table 4Antibiogram of mastitis associated isolates

Name of antibiotics	No. of isolates sensitive	Percentage of sensitivity
Gentamicin	35	84.63
Enrofloxacin	22	72.79
Norfloxacin	21	70.16
Kanamycin	16	68.84
Sulphamethoxazole + Trimethoprim	15	67.53
Oxytetracycline	11	65
Amoxicillin	09	60.16
Penicillin	13	59.64
Colistin	08	57.10
Neomycin	06	54.37
Streptomycin	05	38.16
Chloramphenicol	03	28.85

4. <u>DISCUSSION</u>

This study found the prevalence of subclinical and clinical mastitis in camel, where quarter basis results were (14.28%). Abdurahman and Bornstein (1991) also declared in Jijiga a similar prevalence while a higher rate (47.3%) of CMT result in Afar was reported by Taketelew and Bayleyegn (2001). The penetrating and non-penetrating udder lesions and superficial skin lesions of the teat and udder were noted in camels. The lesions on udder has been reported 100 percent in 10 mastitic camels. Similarly, Teketelew and Bayleyegn (2001) described a high existence of clinical mastitis (72.2%) in camels with udder lesion. In another study, it was reported that the udder or teat skin cuts can be produced by thorny dessert plants that can lead to mastitis. The microbial growth of the udder during mastitis could be directly stimulated by any physical trauma or injury (Woubit et al., 2001). Radostits et al., (2000) reported that the incidence of mastitis could be higher during the period of early lactation and the first phase of dry period.

The present study also showed that Streptococcus and Staphylococcus species were the leading bacterial pathogens for high existence of mastitis in the study zone. Our results are in lineas reported by (Biffa et al., 2005 and Almaw et al., 2008). However, the milk samples of healthy camels showed the prevalence of E. coli between 1.0 and 17.3 % (Abdel Gadir et al., 2005). Prevalence of E. coli in the literature in reported lower than the results of this study. Gram +vecocci have been regarded as the most important source of mastitis in camels and their role in mastitis has been recognized about 93.8%. This report supports and agrees with that described by Woubit et al. (2001). Coagulase negative staphylococci (CNS) among all the bacterial pathogens was found as the major causative agent of mastitis in the dromedary camels. This result settles with that described earlier by other investigators (Abdurahman, 2006; Guliye, et al., 2002). Staphylococcus aureus and Streptococcus agalactiae has been described as the top common sources of mastitis in dromedary camel at Kenya (Younan, 2004) and Eastern Sudan (Obeid, et al., 1996). The commonly isolated bacterial genera from camel mastitis includes Streptococcus, Staphylococcus, Bacillus, Escherichia and Corynebacterium, in this study approve with Sena et al. (2001), Younan et al. (2001), Bekele and Molla (2001), Abdurrahman (2006), Matofari et al. (2005), Abera et al. (2010a) and Kalla et al. (2008) found Staphylococcus, Escherichia and Streptococcus as main pathogens Radostits et al. (2000) observed that S. aureusspreads to healthy animals during milking, because this has capability to grow inside the udder and create a minor subclinical contagion in prolonged period. Regarding findings of this work, this is suggested that for decreasing the possibilities of occurrence of transmissible mastitis in the expanse, the treatment of clinically infected camel, may improve milking hygiene, discarding of lingering mastitis carriers, and prevention of skin lesions should be practiced.

This study has observed in vitro antimicrobial susceptibility of different isolates recovered from clinically and subclinically mastiticmilk samples. The highest percent susceptibility (84.63%), was noticed against gentamycin group. In terms of sensitivity test; it was found that, Staphylococcusaureus isolates were 100% susceptible to tetracycline, kanamycin, chloramphenicol, gentamicin and streptomycine. However tetracycline were observed with good potential for Streptococcus agalactia. Similarly, E. coli isolates were found highly sensitive to kanamycin and gentamicin (Gadir et al. 2005). He also showed that Oxytetracycline, tetracycline and chloramphenicol were effective against camel mastitis pathogens. This profile is somewhat different from that found by Mody (1999), where

he exhibited antimicrobial drugs against different bacterial isolates and found Gentamycin sensitivity against 35samplesand Chloramphenicol 3samples. While moderate susceptibility of antimicrobial drugs was shown by Oxytetracycline (11samples), and Chloramphenicol (3samples), Streptomycine (5samples), Amoxicillin (9samples) and Colistin (8sample).

5. CONCLUSIONS

This study concludes the mastitis was predominant in camels, main pathogens identified were bacteria. Study reveals inadequate management protocols in study area.

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