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Epidemiology of Subclinical Mastitis in Dromedary Camels (*Camelus dromedarius*) of Two Distinct Agro-Ecological Zones of Pakistan

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MA, MI, MC and RH designed the study. MA, AIA, NUK, MK, MAK, MA and MSH did the sampling and field work. IQ, GR, and IH did the lab work. MLS and IK did the statistical analysis and wrote the article.

Key words
Camel, Subclinical mastitis, Risk factors, *Staphylococcus aureus*, Cholistan desert area, Suleiman mountain range.

ABSTRACT

The present study was accomplished to explore the epidemiological dynamics of subclinical mastitis, caused by *Staphylococcus aureus*, in dromedary camels (*Camelus dromedarius*) reared in two distinct agro-ecological zones of Pakistan *viz*. Cholistan desert and Suleiman mountain range. A total of 768 lactating she-camels were screened for subclinical mastitis using California mastitis test (CMT). A preformed questionnaire was used to obtain data for the potential risk factors. Results showed an overall prevalence of 47.14% for subclinical mastitis. Statistical analysis revealed non-significant (*P>0.05)* difference between Cholistan desert and Suleiman mountain range (48.18% and 46.09%, respectively). *S. aureus* was isolated from 53.03% of the milk samples, with significantly higher (*P<0.05)* prevalence in Cholistan desert (66.10%) as compared to Suleiman range (57.98%). Blind quarter prevalence of *S. aureus* subclinical mastitis was 8.46% and 11.59% *(P<0.05)* in Cholistan desert and Suleiman range, respectively. The non-parametric statistical analysis showed significant association *(P<0.05)* of thin body condition, older age, lack of teat dipping and higher parity number with *S. aureus* subclinical mastitis. This study highlights the effect of agro-climatic conditions on *S. aureus* subclinical mastitis which is instrumental when devising strategies for effective disease control according to the climatic conditions of the area.

INTRODUCTION

Mastitis, an inflammatory disease of udder, in all of its manifestations is health and production compromising disease. The economic losses due to mastitis are in the form of milk deterioration, treatment costs, low milk prices due to quality deterioration with increased somatic cell count, higher labor involved in animal treatment and higher culling rates. A single clinical case was estimated to cause USD 21$ loss (Sinha *et al*., 2014) and is listed among the diseases causing the maximum economic losses (Fareed at al., 2016). Subclinical form of mastitis is thought to be more than 70% of all types of mastitis manifestations (Abebe *et al*., 2016). The single quarter infection due to major mastitis pathogen was noted to cause 30% reduction in milk production (Jones and Bailey, 2009).

The prominent bacterial causes of camel mastitis

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include *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *E. coli* (Aqib *et al*., 2017b). *S. aureus* is the major pathogen that can induce clinical or subclinical mastitis with huge economic losses (He *et al*., 2016). This pathogen alone accounts for 20.35% prevalence in the world where as Pakistan observes 52.3% (Ahmad *et al*., 2012; Sarwar, 2013). Its presence in the micro or macro-environment causes serious intra-mammary infection (Radostits *et al*., 2007), impairing alveolar physiology by destroying alveolar and ductal cells. These damaged cells may combine with leukocytes to clog the milk ducts that drain the alveolar areas, contributing to further scar tissue formation, occlusion of ducts, and decreased milk production (Mbuk *et al*., 2016). *S. aureus* executed intramammary infections are chronic because the bacteria avoids killing by hiding in neutrophils and other host cells (Petersson-Wolfe *et al*., 2010).

The camel is well adapted to arid and hot environment and is spread across 47 countries (FAO, 2001) blessed with dairy characteristic holding maximum milk production of 40 liters per day (Faye and Bonnet, 2012). The population is increasing at faster rate (Nagy *et al*., 2013) with ten times higher milk consumption than to that of meat and has gained access to markets of Gulf countries (El-Agamy and Khatab, 1992). The camel udder has been reported to be suffering from mastitis in various parts of world. To the dismay of this species, epidemiological studies are scarce despite of recent reports published with emphasis on this ailment. The estimation of prevalence and its associated risk factors is inevitable for preventive strategies. Bearing in mind the importance of camel mastitis, the current study was planned to investigate the status of *S. aureus* caused subclinical mastitis in distinct agro-ecological zones of Pakistan.

MATERIALS AND METHODS

Study area

Cholistan desert is located in the South–West of Punjab, Pakistan, having an area of 26,000 sq. kms. It is located between latitudes of 27° to 42° and 29°N and longitudes of 57° to 60°E. Cholistan desert is home to 11328 camels, providing the only source of income to the marginalized desert dwellers (Ali *et al*., 2009). The Suleiman mountain range is located at 29°37'-31°70' and 68°06'-70°20' at North latitudes and East longitude, 600-1350 meters above the sea level. Temperature reaches 40°C in summers while drops below freezing point in winters (Wikramanayake, 2002).

Sample size

Sample size was calculated by non-probability convenient sampling method (Thrusfield, 2007);

$$n = Z^2 P_{exp} (1 - P_{exp})/d^2$$

Where, n is required sample size, P_{exp} is expected prevalence and d is desired absolute precision, keeping in view 50% of prevalence with 95% confidence interval.

A total of 768 dromedary camels reared by farmers and pastoral families in Cholistan desert (n=384) and Suleiman Range (Barkhanand Tribal Area Dera Ghazi Khan, n=384) were included in this study.

Risk factors analysis

A dichotomous type questionnaire was constructed to find out risk factors to gather data about teat dipping, teat abnormality, age, parity, and lactation stage, rearing system, water source, tick infestation, feeding system, and body condition of animal.

Screening for subclinical mastitis

Lactating camels were screened for subclinical mastitis using California Mastitis Test (CMT) kit (Cat. No. 170361, Bovi-Vet, Kruuse, Denmark) as described by (Schalm *et al*., 1971). The camels, positive for subclinical mastitis through CMT were selected for isolation and identification of *S. aureus*.

Isolation and identification S. aureus

Milk samples were aseptically collected from positive quarters for isolation and identification of *S. aureus*. Briefly, 2mL of CMT positive milk sample was centrifuged at 2000 g for 10 min. The sediment was streaked on to blood agar (Columbia agar base having included 5% defibrinated sheep blood) and was incubated for 24 to 48 h at 37°C (Cruickshank *et al*., 1975). The colonies grown on blood agar were sub-cultured on mannitol salt agar. The typical round colonies were projected for Gram's staining, catalase test and coagulase test to identify *S. aureus* as per directed by Bergey's Manual of Determinative Bacteriology (Buchaman and Gibbons, 1974).

Statistical analysis

Prevalence of mastitis and bacterial isolates were calculated as described by Thrusfield (2007). The association of risk factors with mastitis was determined by Pearson's chi square test at 95% confidence interval ($P<0.05$) using SPSS version 22 (SPSS, 2013).

RESULTS

Overall CMT based prevalence of subclinical mastitis was 47.14% in camels of Cholistan desert and Suleiman mountain range. A non-significant difference *(P>0.05)* was observed between study areas. Prevalence

of subclinical mastitis in Yazman area of Cholistan desert was the highest (49.79%) followed by Rakni and Tribal area of Suleiman range presenting 47.15% and 45.59% respectively. Interestingly, prevalence of *S. aureus* from subclinical mastitis samples differed significantly *(P<0.05)* among different areas within each distinct zone whereas no significant difference was recorded between two distinct study areas on collective prevalence basis (Table I). The overall *S. aureus* prevalence was found 53.03% from this study with higher prevalence *(P<0.05)* recorded in Cholistan desert (57.84%) area followed by Suleiman mountain range (48.02%).

Table I.- Prevalence of subclinical mastitis and *Staphylococcus aureus* prevalence in two distinct zones of Baluchistan and Punjab.

Study Zones	Mastitis prevalence on the basis of CMT			*S. aureus* prevalence from subclinical mastitis samples		
	Prevalence (%)	CI (95%)	p-value	Prevalence (%)	CI (95%)	p-value
Suleiman range (Baluchistan)						
Tribal area	119/261 (45.59)	39.4-51.8	0.75	69/119 (57.98)	47.3-65.5	0.000
Rakni	58/123 (47.15)	38.1-56.4		16/58 (27.59)	18.3-44.3	
Total Suleiman mountain range	177/384 (46.09)	41-51.2	0.563	85/177 (48.02)	41-56.2	0.061
Cholistan desert (Punjab)						
Yazman	118/237 (49.79)	43.3-56.3	0.422	78/118 (66.10)	56.8-74.6	0.003
Khair Pur Tamaywali	67/147 (45.58)	37.4-54		29/67 (43.28)	28.5-53	
Total Cholistan desert area	185/384 (48.18)	43.1-53.3		107/185 (57.84)	50.4-65	
Grand Total	**362/768 (47.14)**	**43.6-50.7**	**-**	**192/362 (53.03)**	**48-58.6**	

P<0.05 indicates significant results.

Table II.- Association of determinants with subclinical mastitis in two distinct zones of Baluchistan and Punjab.

Parameters	Levels	Cholistan desert area			Suleiman mountain range		
		No. of cases positive	CI (95%)	P-value	No. of cases positive	CI (95%)	P-value
Teat dipping	Yes	1/40 (2.5)	0.063-13.2	0.001	4/43 (9.3)	2.6-22.1	0.001
	No	184/344 (53.5)	48.1-58.9		173/341 (50.73)	45.3-56.2	
Teat abnormality	Normal	165/355 (46.5)	41.2-51.8	0.020	169/362 (46.7)	41.5-52	0.346
	Injured	20/29 (69.0)	49.2-84.7		8/22 (36.4)	17.2-59.3	
Age group	4-7 year	44/114 (38.6)	23.2-38.8	0.032	41/112 (36.6)	27.7-46.2	0.016
	8-10 year	94/187 (50.3)	42.9-57.6		74/161 (46.0)	38.1-54	
	>11 year	47/83 (56.6)	45.3-67.5		62/111 (55.9)	46.1-65.3	
Parity number	1-2	93/217 (42.9)	36.2-49.7	0.001	52/138 (37.7)	29.6-46.3	0.007
	3-4	74/148 (50.0)	41.7-58.3		110/225 (48.9)	42.2-55.6	
	5 and above	18/19 (94.7)	74-99.9		15/21 (71.4)	47.8-.88.7	
Lactation stage	1-4 months	93/214 (43.5)	36.7-50.4	0.068	65/137 (47.4)	38.9-56.1	0.684
	5-7 months	51/88 (58.0)	47-68.4		83/177 (46.9)	39.4-54.5	
	8-12 months	41/82 (50.0)	38.7-61.3		29/70 (41.4)	29.8-53.8	
System of rearing	Nomadic	61/126 (48.4)	39.4-57.5	0.949	27/75 (36.0)	25.2-47.9	0.51
	Seminomadic	124/258 (48.1)	41.8-54.3		150/309 (48.5)	42.8-54.3	
Water source	Pond	Pond water was only source seen			68/145 (46.9)	38.6-55.4	0.417
	Canal				89/203 (43.8)	36.9-51	
	Underground water				20/36 (55.6)	38.1-72.1	
Tick infestation	Yes	113/241 (46.9)	40.5-53.4	0.512	92/217 (42.4)	35.7-49.3	0.098
	No	72/143 (50.3)	41.9-58.8		85/167 (50.9)	43.1-58.7	
Feeding system	well fed	60/156 (38.46)		0.191	65/145 (44.83)		0.697
	Underfed	125/228 (54.82)	48.1-61.4		112/239 (46.86)	40.4-53.4	
Body condition	Normal	54/183 (29.51)	23-36.7	0.001	42/175 (24)	17.9-31	0.001
	Thin	131/201 (65.17)	58.2-71.7		135/209 (64.59)	57.7-71.1	

Table III.- Prevalence of blocked quarters of She camel due to mastitis in two distinct zones of Baluchistan and Punjab.

Quarters	Cholistan			Suleiman Range			p-value between Cholistan and Suleiman Range
	Prevalence (%)	95% CI	p-value	Prevalence (%)	95% CI	p-value	
FR	51/384 (13.28)	10.1-17.1	0.022	43/384 (11.20)	8.2-14.8	0.022	0.378
FL	35/384 (9.11)	6.4-12.4		28/384 (7.29)	4.9-10.4		0.357
RR	57/384 (14.84)	11.4-18.8		38/384 (9.89)	7.1-13.3		0.037
RL	35/384 (9.11)	6.4-12.4		21/384 (5.47)	3.4-8.2		0.057
Total	178/1536 (11.59)	10-13.3		130/1536 (8.46)	7.1-10		0.004

Values in parenthesis are percentages. FR, front right; FL, front left; RR, rear right; RL, rear left. P<0.05.

Table IV.- Quarter based prevalence of subclinical mastitis in two distinct zones of Baluchistan and Punjab.

Quarters	Cholistan			Suleiman Range			p-value between Cholistan and Suleiman Range
	Prevalence (%)	95% CI	p-value among quarters	Prevalence (%)	95% CI	p-value among quarters	
FR	141/333 (42.34)	37-47.8	0.022	135/341 (39.59)	34.4-45	0.14	0.467
FL	126/349 (36.10)	31.1-41.4		121/356 (33.99)	29.1-39.2		0.556
RR	145/327 (44.34)	38.9-49.9		132/346 (38.15)	33-43.5		0.103
RL	120/349 (34.38)	29.4-39.6		115/363 (31.68)	26.9-36.7		0.443
Total	532/1358 (39.18)	36.6-41.8		503/1406 (35.78)	33.3-38.3		0.065

Values in parenthesis are percentages. FR, front right; FL, front left; RR, rear right; RL, rear left. P<0.05.

The chi square test showed that teat dipping, age group, parity number and body condition are significantly associated *(P<0.05)* with subclinical mastitis (Table II). Teat abnormality was significantly *(P<0.05)* associated with mastitis in Cholistan while no such association *(P>0.05)* was noticed in animals of Suleiman range. The determinants like camel rearing system, water channels source, tick infestation and feeding system presented no association *(P>0.05)* with subclinical mastitis.

Table III shows significant *(P<0.05)* higher prevalence of blind quarters in Cholistan area (11.59%) as compared to Suleiman range (8.46%). The prevalence of rear right (RR) blockage differed significantly *(P<0.05)* between the two study areas. The prevalence of blocked quarters within each zone was found significantly different *(P<0.05)* among all four quarters. The highest prevalence was noticed in case of rear right (RR) (14.84%) from Cholistan desert while front right (FR) (11.20%) in Suleiman range.

DISCUSSION

This study was conducted to determine the influence of climate on the prevalence of *S. aureus* caused subclinical mastitis in camels of distinct ecological zones of Pakistan. Results showed astonishingly higher rates of prevalence in study areas. Findings of the current study was in line with the previous reports of Aqib *et al*. (2017b) and Ahmad *et al*. (2012) who reported 41.67% and 46% prevalence of subclinical mastitis, respectively, and Abdulkadhim (2012) and Wanjohi *et al*. (2013) from Kuwait and Kenya reporting 43% and 60%, respectively. Contrary to these, lower prevalence was noticed by Abera *et al*. (2010), Abdurahman (2006) and Bekele and Molla (2001) which can be attributed to variation in geographical area, rearing practices and hygienic farm management. Ahmad *et al*. (2012) reported 42.19% prevalence of *S. aureus* in Pakistan. The higher percentage of *S. aureus* can be related to poor farm management and unhygienic milking practices that might have led to robust spread of this contagious pathogen in camels (Radostitis *et al*., 2007).

The significant association of determinants like teat dipping, age group, parity number and body condition with mastitis was found in line with findings of Aqib *et al*. (2017a), Husein *et al*. (2013) and Ahmad *et al*. (2012), whereas rearing system, water channel source, tick infestation and feeding system determinants of current study contradicted with findings of aforementioned studies. This variation is may be because of better rearing practices system in areas where camel rearing is sole business. The poor udder hygiene, under feeding and thin body condition provide favorable environment to bacterial pathogenesis development (Mbuk *et al*., 2016). Tying the teat of camel with string to avoid calf suckling is common practice among camel rearing masses, which injures the udder and favors bacterial entry into teat (Woubit *et al*., 2001). The dry animals are more prone to mastitis because

of late formation of keratin plug at teat, reduced leukocyte, and diluted lactoferrin, inactivation of immune system and lack of flushing activity (Smith *et al*., 1985).

Quarter based prevalence of subclinical mastitis was found to be coherent with the results reported by Ahmad *et al*. (2012), whereas lower rates were reported by Aqib *et al*. (2017a). The discrepancy in results of various studies lies in overall prevalence status and associated risk factors of research area. Lack of sanitary conditions at farm and unhygienic situation during milking led to higher prevalence rate (Shittu *et al*., 2012). The higher infection status in right quarter side is suspected because of left sided milking that may spread infection to other side of quarter because of contaminated milking setup (Shittu *et al*., 2012). The higher teat blockage in current study might be because of untreated cases due to unavailability of veterinary services in remote areas and irrational treatment approaches.

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Compliance with ethical standards

The authors state that except for primary sample collection there was no use of animals throughout this research work and all the respondents gave consent for questionnaire.

Statement of conflict of interest

Authors declare no conflict of interest

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