



## Prevalence of Mastitis and its Impacts on Milk Composition in Lassi Camel Breed in Lasbela, Pakistan

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### Abstract

The current study was done to evaluate prevalence of mastitis in Lassi camel breed in Lasbela, Pakistan. A total of 100 camel cows were selected for milk sampling for isolation and identification of mastitis causing bacteria and biochemical tests. A questionnaire was used to know prevalence of mastitis associated with species, breed, age, parity, and udder hygiene condition, frequency of milking, practice of teat dipping, and feeding frequency of the camel cows. A t-test was used to analyse the results and prevalence data was expressed in percentage. The total 43% prevalence of mastitis was observed in Lassi camel of ofLasbella district Balochistan, Pakistan. It was observed that prevalence of mastitis 38%, 41.18%, and 47.5% was increased with advancement in the age 4-7, 7-10, and above 10 years, respectively. It was also observed that some associated factors such as milking and feeding frequency, and udder hygiene play important role in mastitis. During mastitis, significantly increased in fat, protein, total solids, solids not fat, and moisture contents, and pH and significantly declined in lactose indices was observed. Staphylococcus aureus species were dominantly found followed with Streptococcus agalactia and Streptococcus dysagalactia. This study will helpful to the local farmers and veterinarian regarding proper use of antimicrobial agents, and maintenance of udder hygiene to control mastitis in Lassi camel breed in Lasbela, Pakistan.

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**Keywords: Lassi, Camel, Mastitis, Prevalence, Milk**

### Introduction

There are two types of camel, the Bactrian (*Camelus bactrianus*) or double humped and the dromedary (*Camelus dromedaries*) or single humped. The globe livestock camel population is estimated to be around 40 million and 1.1 million in Pakistan with 9.4 million, the highest camel population in Chad. Pakistan is placed in top 10 in camel population (Worldstats, 2023). Camel is known for multipurpose and can survive in hot conditions of dessert, arid and semi arid regions

where browse and water are limited (Bornstein and Younan, 2013). In Asia and Agrican countries, camels contributed in milk production with estimaton of 3.15 million tons by FAO 2020 (Nagy et al., 2022). There are two types of camels including riverine and mountain camels in Pakistan. Both are single humped in Pakistan. The first ones are found in coastal areas, deserts and irrigated plains and other ones are found in mountain areas (Faraz et al., 2013).

The Lassi camel thrives in the coastal areas of Balochistan and Sindh, where the climate is

characterized by high temperatures and humidity. This versatile camel serves not only as a means of transportation but also forms distinct herds, playing a central role in the region's livestock activities (Kakar et al., 2009).

The milk of the dromedary camel holds considerable importance, similar to other dairy animals, it is susceptible to mastitis. This complex ailment is prevalent globally in dairy animals, causing substantial economic losses primarily due to both clinical and subclinical mastitis, the latter demanding indirect diagnostic methods (Geresu et al., 2021). Camel milk isposes good medicinal properties against diabetes, jaundice, and asthma, and nutritional characteristics such as protein, fat, minerals and vitamins, especially vitamin C (Kumar et al., 2016). Due to these properties the demand of camel milk increasing indigenously and internationally, and finally contribution in economy (Gossner et al., 2016). Camel is also used as a source of meat and draft purpose (Abera et al., 2016).

From conclusive data, 45.66% of camel population infected with mastitis (Aqib et al., 2022). It is well established that *Staphylococcus aureus* alone accounts for 20.35% mean prevalence of mastitis in the world, and 52.3% in Pakistan (Ahmad et al., 2012; Sarwar, 2013). Mastitis is complicated disease etiological including various microbes, while bacteria is main cause of mastitis in animals (Klaas et al., 2018). It is reported that *Candida* is genus of fungi cause of mycotic mastitis (Dworecka-Kaszak et al., 2012). It is well established that various causative pathogens including bacterial mastitis (*Staphylococcus* and *Streptococcus*) (Abera et al., 2016, Alebie et al., 2021, He et al., 2016, El Tigani-Asilet et al., 2020). Mycotic mastitis is caused by *Candida*, *Cryptococcus*, *Trichosporon* and *Aspergillus* (Al-Dughaym et al., 2015). It is well established about the prevalence and presence of mastitis in camel in various countries including Pakistan (Aqib et al., 2022).

Balochistan province is home of camel farming and fewer studies were reported regarding prevalence of mastitis in camel. The present study investigate the prevalence of subclinical and clinical mastitis and associated factors in local Lassi camel, and furthermore impact of mastitis on milk composition.

## Materials and Methods

### Study Location

#### Lasbella District Geography and Climate

Lasbella is the seventh largest district of Balochistan, with an area of 15,153 square kilometers. The district lies at 65 degree, 12, 11 to 67 degree, 25, 39 East longitudes and 24 degree, 53, 2 to 26 degree, and 39, 20 North latitude. The district has five Tehsils and twenty two union councils. Lasbellais at 1075 kilometer distance from the capital city of Islamabad and 116 North-West from Karachi. The climate in Lasbella has hot, dry tropical desert climate. It is only a few degrees north of tropical cancer. Although the sea winds have reduced the weather significantly over interior Balochistan, where it is 50 °C (122 °F) in summer.

### Study Design

A cross sectional study was conducted for the period of four months (August 2019 to November 2019). Prevalence and occurrence of clinical and sub clinical mastitis was via single visit accordance with multiple-subject survey (ILCA, 1990). Sampling frame was constructed using purposive sampling method (Petrie and Watson, 2013). The total 100 camel cows (small, medium and large scale farmers) were monitored for the presence of clinical and sub clinical mastitis.

The informations from farmers using designed questionnaire performa including species, breed, age, parity, and udder hygiene condition, frequency of milking, practice of teat dipping, and feeding frequency of the camel cows, were collected. Camels were owned by pastoralists comprising of semi-nomadic and sedentary camel production system. The following criteria were used for selection of camels:

- Same nutritional status
- Different management conditions
- Willingness of the pastoralists to participate in the study
- Accessibility of the site, so that samples collected could be immediately transferred to diagnostic laboratory for further analysis.

### Mastitis Diagnosis and Confirmation

Firstly mastitis was diagnosed on the basis of clinical signs (redness, swelling, hotness and pain in udder tissues). Later confirmed with surf field mastitis test (SFMT) and strip cup test according to previously reported protocols (Karabasanavar et al., 2021) and KerroDego and Tareke, 2003). The protocol. Prevalence of mastitis was calculated accordance with previous reported method (Barlow, 2009).

### Collection of Milk Samples

Milk samples were collected from confirmed cases of clinical and sub clinical mastitis for bacterial identification and milk chemical composition analysis. Milk samples were collected aseptically (70% methyl alcohol) in sterilized glass tubes. First few strips of milk discarded and then 10 ml milk sample collected from each teat. The milk samples were collected and stored in ice boxes transferred to laboratory of Lasbela University of Agriculture, Water, and Marine Sciences for bacteria identification and milk composition analysis.

### Isolation and Identification of Bacteria

Each labeled sample was streaked and cultured on nutrient media according to reported protocol (National Mastitis Council, Inc., USA, 1990). Morphology of bacterial colonies were observed and gram's staining was performed according to previous reported method (Smith et al., 2005). Later, the colonies were sub cultured on selective medium (manitol salt, and blood agar) for the growth of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysagalactia*. Then morphological characteristics of bacterial colonies of individual species were observed.

### Biochemical Tests of Bacteria

The biochemical tests were performed for further confirmation the *Staphylococcus aureus* characteristic. These biochemical tests includes catalase test, methyl red reaction, citrate utilization, urease test, oxidase test, test of nitrate reduction, and H<sub>2</sub>S production test, and were performed according to reported protocol (National Mastitis Council, Inc., USA, 1990).

### Milk Composition Analysis

The milk composition analysis was performed according to previous documented methods (Aoac, 1990). The milk composition parameters included were total solids, total fats, solid not fats, total protein, non-casein nitrogen, non-protein nitrogen, total ash, and lactose.

### Statistical Analysis

Data were presented as mean  $\pm$  SD. A t-test was used with  $P \leq 0.05$  as significance level. Prevalence data was expressed in percentage (Daniel, 2010).

## Results

### Prevalence of Clinical and Sub Clinical in Lassi Camel Cow

A total of 100 camel cows were monitored for the presence of clinical and sub clinical mastitis. **Table -1** depicts the overall prevalence of mastitis

i.e. 43 Percent (43/100). Data on prevalence of clinical & sub clinical mastitis was determined as, 5 % (5/100) and 38 % (38/100) respectively.

### Associated Factors of Prevalence of Mastitis in Camel Cow

The information collected from the farmer on questionnaire included the associated factors (Age, parity, milking frequency, teat dipping, quantity of nutrition, udder hygiene) involved in the occurrence of mastitis.

#### Age wise prevalence

The 47.5 % prevalence was observed in age group 10 years and above, followed by 41.18% in 7 to 10 years and 38% in 4 to 7 years age group, respectively. Lowest prevalence was observed in below 7 year age group shown in **Table-2**.

#### Parity

Highest prevalence (57.9%) was observed animals at 4<sup>th</sup> parity followed by 1<sup>st</sup> parity (51.85 %), 3<sup>rd</sup> parity (42.86 percent) and 2<sup>nd</sup> parity (23.07 %), respectively. The prevalence of mastitis in 2<sup>nd</sup> parity was lowest (23.07 %) shown in **Table 3**.

#### Milking Frequency

The effect of milking frequency on the prevalence of mastitis in Lasi camel cows was also evaluated. The highest prevalence (46.67 %) of mastitis was observed in the camel cows milked out once per day as compared with the milked out more than once (10 %) shown in **Table 4**.

#### Teat Dipping

The highest prevalence (48.28 %) was observed in camel cows without teat dipping vs. treated with teat dips (7.7 %) shown in **Table 5**.

#### Quantity of nutrition

The highest prevalence was observed in underfed camel cows (65 %) followed by well-fed group 10 % shown in **Table 6**.

#### Udder Hygiene

The highest prevalence was observed in the camel cows with unsatisfactory udder condition (50 %) followed by cows with satisfactory udder condition (18 %) in Lassi camel cows shown in **Table 7**.

### Prevalence and Identification of Bacterial Isolates

*Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysagalactiae* bacterial species were confirmed via morphology, staining, and biochemical. The most predominant

isolate was *Staphylococcus aureus* (53.49 %) followed by *Streptococcus agalactiae* (27.90 %) and *Streptococcus dysagalactiae* (18.60 %) shown in **Table 8**.

#### Milk Analysis of Mastitis and Normal Milk

Mastitic vs. normal milk composition shown in Table-4. As compared to normal milk significantly ( $0.05 < P$ ) increased in fat

( $6.52 \pm 0.57\%$ ), protein ( $6.24 \pm 0.22\%$ ), total solids ( $16.36 \pm 0.34\%$ ), solids not fats ( $12.85 \pm 0.10\%$ ) moisture content ( $89.74 \pm 0.40\%$ ) and pH value ( $6.86 \pm 0.02$ ) was observed. Ash content was non-significantly ( $1.15 \pm 0.31\%$ ) increased. Lactose content ( $2.62 \pm 0.09\%$ ) was significantly low in mastitic milk samples than normal milk samples ( $7.80 \pm 0.2\%$ ) shown in **Table 9**.

**Table 1: Prevalence of clinical and sub clinical mastitis in lassi camel cows in Lasbella Balochistan**

No Of Camel Examined	No Of Camels Positive	Over all Prevalence Percent	No of Positive cases for SCM	Prevalence Per cent%	No of positive cases for CM	Prevalence percent
100	43	43	38	38	5	5

SCM: Sub clinical mastitis, CM: Clinical mastitis

**Table 2: Age wise prevalence of mastitis in Lassi camel cows in Lasbela, Balochistan**

Age group	Number of animals	Number of positive	Number of positive SCM cases	Number of positive clinical cases	Prevalence %
4-7 year	26	10	10	0	38.41
7-10 year	34	14	12	2	41.17
10 & above year	40	19	16	3	47.5

**Table 3: Parity prevalence of mastitis in Lasi camel cows in district Lasbella, Balochistan**

Parity Number	Number of examined	Number of positive	Number of positive subclinical	Number of positive clinical	Prevalence %
1	27	14	11	3	51.85
2	26	6	6	0	23.07
3	28	12	11	1	42.85
4 and Above	19	11	10	1	57.89

**Table 4: Prevalence of mastitis on the basis of milking frequency in Lassi camel cows in Lasbella**

Frequency of Milking	Number of animals were examined	Number of positive animals	Prevalence per cent
Once	90	42	46.67
More than once	10	1	10

**Table 5: Prevalence of mastitis on the basis of teat dipping performance in Lassi camel cows in district Lasbella**

Teats dipped with antiseptics	Number of animals were examined	Number of positive animals	Prevalence per cent (%)
Performed	13	1	7.7
Not performed	87	42	48.28

**Table 6: Prevalence of mastitis in Lasi camel cows on the basis of quantity of nutrition in Lasbella district**

Feeding	No of camels examined	Number of positive	Prevalence percent
Well fed	40	4	10
Under fed	60	39	65

**Table 7: Prevalence of mastitis in Lassi camel cows on the basis of udder hygiene**

condition of Udder hygiene	Number of animals were examined	Number of of positive animals	Prevalence per cent (%)
Good Condition for udder hygiene	22	4	18.0
Not Good Condition for udder hygiene	78	39	50.0

**Table 8: Prevalence of bacterial isolates**

Organism	Number	Percentage
<i>Staphylococcus aureus</i>	23	53.49
<i>Streptococcus agalactiae</i>	12	27.90
<i>Streptococcus dysagalactiae</i>	08	18.60

**Table 9: Comparison of chemical parameters of mastitic and normal milk samples Lassi camel milk from Lasbella district (n= 100)**

Parameters	Mastitic milk samples	Normal milk samples	P-value
Fat	6.52±0.57	3.44±0.21	0.0000
Protein	6.24±0.22	2.66±0.11	0.0001
Lactose	2.62±0.09	7.80±0.2	0.0000
Total	16.36±0.34	10.63±0.17	0.0000
SNF	12.85±0.10	8.28±0.22	0.0000
Ash	1.15±0.31	0.63±0.02	0.2858
Moisture	89.74±0.40	81.24±0.18	0.0000
pH	6.86±0.02	6.69±1.00	0.0000

## Discussion

The dromedary camel (*Camelus dromedarius*) is the most important livestock animal in the desert and semi desert areas of Northern and Eastern Africa, Asia and in the deserts of the Arabian Peninsula (Faye, 2014). Clinical mastitis in camels can be easily recognized, subclinical mastitis almost always passes unnoticed. This is due to its high prevalence in many countries among lactating camel herds (Abera, et al., 2016). Mastitis risk factors or disease determinants can be classified into three groups: host, pathogen and environmental determinants including management. Bacteria, which can cause mastitis, are present in air, water and other surfaces in the environment including human and animal skin. *Streptococcus agalactiae*, other *Streptococcus spp.*, *Staphylococcus aureus*, coagulase-negative staphylococci, and *Escherichia coli* are incriminated as the major bacterial causes of mastitis in the camel (Aqib et al., 2017).

In current study, the average prevalence of mastitis was 43% (43/100) in Lassi camel. Lassi camel cows 38 percent % the incidence of subclinical mastitis was high in (38/100) compared to 5 percent % (5/100) of clinical mastitis. The present study relates with the results of following researchers of the globe, for example,

according to Abdurahman, (2006) average prevalence of camel mastitis lies at about 46.5% in the world. The lowest percentage of prevalence studied is said to be near to 22%. In Sub-Saharan regions like Sudan the occurrence of mastitis in camel population is noted to be 66% studied by (Obied et al., 1996). In Borena, the Southern part of Ethiopia, the prevalence of mastitis is recorded as high as 74%. North Eastern Ethiopia was recorded, having a about 59.8% of mastitis in the camel cows of the region (Husein et al., 2013).

*Staphylococcus aureus* (53.49 percent) followed by *Streptococcus agalactiae* (27.90 percent) and *Streptococcus dysagalactiae* (18.60 percent) was the most dominant isolate according to traditional bacterial isolates. The isolates mentioned above have been identified by many studiers from the world acting as causative agents of camel cow mastitis. Bacterial isolates of camel's milk having mastitis were *Staphylococcus aureus* (53.49%) followed by *Streptococcus dysagalactiae* (18.60) & *Streptococcus agalactiae* (27.90%) (Younan et al., (2013). This study was carried out in regions of Kenya & Sudan. Similarly, in camel mastitis the share of *Staphylococcus aureus* stood at 52.3% in the study from Saudi Arabia. Other bacterial isolates like *Streptococcus*, *E.coli*, and *Bacillus* were also

found causing mastitis in camel cows (Radostits, et al. 2007).

The highest prevalence (47.5 percent) was determined in the age group of 10 and above, followed by (41.18 percent) in the age group of 7-10 years and (38 percent) in the age group of 4-7 years, respectively. The maximum prevalence was shown in the age group of 4 and above (57.9 percent), followed by 1st parity (51.85 percent), 3rd parity (42.86 percent) and 2nd parity (23.07 percent). The prevalence of 2nd parity in Lasi camel cows was lowest (23.07 %). The results of the study on the age and parity factor are similar & according to the study of Sibtain et al., (2012) and (Durrani et al. 2017) from Pakistan.

It was reported about 80% of camel mastitis in age group 14-16 years while 33.3% mastitis in camels aged between 5-7 years mastitis. Whereas, Abera et al., (2016) reported that 75% of mastitis cases in camels were age b/w 4-7 years 91.43% in camels aged 7-10 years and 94.44% mastitis at 10 years of age. In another study conducted by Shittu et al., (2012). the study revealed that aged animals were more prone to mastitis than young animals. The occurrence of mastitis in during various stages of lactation like early lactation, mid lactation and late lactation was 39%, 32% and 27% respectively as studied by (Cervinkova et al., 2013).

The highest prevalence (46.67%) of mastitis was observed in the camel cows milked once per day as compared with the milked out more than once (10 percent). Frequent milking and suckling by the calf keeps flushing the mastitis pathogens out but some studies have shown that it is the major cause of mastitis in camels (Aliet al., 2019).

The highest prevalence (48.28%) was observed in camel cows without teat dipping as compared with those being treated with teat dips (7.7%) in Lassi camel cows. These results comparable with findings of Sibtain et al., (2011). observed that she-camels with skin lesions on their teats and/or udder had a high prevalence of mastitis; similar observation has been recorded by Abdurahman, (2006) in eastern Ethiopia. Hertl et al., (2010) found in the Kiambu district of Kenya that mammary gland quarters with teat lesions were 7.2 times more likely to have bacterial organisms isolated from them than those without any teat lesions.

The highest prevalence was observed in underfed camel cows (65%) followed by well-fed group (10%). The highest prevalence was observed in the camel cows with unsatisfactory udder condition (50%) & followed by cows with satisfactory udder condition (18%) in Lassi camel cows. The significant association of determinants

such as teat dipping, age group, parity number and body condition with mastitis was found in line with findings of Aqib et al., (2017). 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 (Oikonomou et al., 2014). The poor udder hygiene, under feeding and thin body condition provide favourable environment to bacterial pathogenesis development. 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 (Ahmad, et al., (2012) 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., 2005). It was recorded that chemical composition of Normal Milk percentage was similar as compare to these results. However, mastitic milk % with milk fat, lactose content were increased and some values were decreased such as solid not fat, total solid, moisture and ash content (Hadeef et al. 2022).

### Conclusions and Recommendations

A high prevalence of mastitis was observed in Lassi camel cows in district Lasbella, Balochistan, Pakistan. Bacteria species including *Staphylococcus aureus*, *Streptococcus dysagalctiea* and *Streptococcus agalactiea* were found. Furthermore, associated factors (milking once/year, teat not dipped, under feeding animals, and unhygienic conditions of udder) were observed which lead towards the occurrence of mastitis. In order to reduce the high prevalence of contagious mastitis in the area, fortnight mastitis control program, improved milking hygiene, prevention of skin lesions, culling of chronic mastitis carriers, and treating of clinically infected she-camels should be practiced. This study will provide guidelines to the local farmers and veterinarian regarding judicious use of antimicrobial agents and udder hygiene to control mastitis in Lassi camel breed in Balochistan, Pakistan

### Novelty Statement

There are a few reported studies about prevalence of mastitis in camel population of Pakistan. Herein we reported first time the comprehensive study about prevalence of mastitis in indigenous (Lassi breed) of camel in District Lasbela, Balochistan, Pakistan.

**Conflict of interest**

All the authors clearly declared no conflict of interest.

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