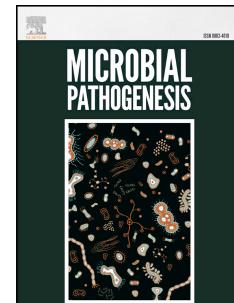


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Epidemiological study of common diseases and their risk factors in camels in South Punjab, Pakistan

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2 **EPIDEMIOLOGICAL STUDY OF COMMON DISEASES AND THEIR RISK**
3 **FACTORS IN CAMELS IN SOUTH PUNJAB, PAKISTAN.**

4

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18 **Abstract**

19 Bacteriological study of mastitis along with common blood protozoan diseases were studied in
20 dromedary camels in Cholistan, Dera Ismail Khan and Rahim Yar Khan districts in South
21 Punjab, Pakistan. For this purpose 300 camels were sampled randomly at different common
22 grazing and watering point. For study of blood parasites clinically suspected and apparently
23 healthy camels, 150 each, were sampled. An overall prevalence of 15% and 5% was recorded
24 for trypanosomiasis and Anaplasmosis respectively. *Trypanosoma evansi* was identified with
25 280 bp product on polymerase chain reaction test. There was significant ($P < 0.05$) decline in the
26 values of total erythrocyte counts, hemoglobin concentration, packed cell volume, serum total
27 proteins and albumin while erythrocyte sedimentation rate was increased in infected camels as
28 compared to healthy ones. Aspartate aminotransferase, alanine aminotransferase, gamma
29 glutamyltransferase and alkaline phosphatase were also significantly increased in blood
30 protozoan the infected animals. Milk samples for bacteriology were collected from healthy
31 lactating camels ($n = 100$). Information about different risk factors were gathered on designed
32 performa. Subclinical mastitis on surf field test was recorded in 42% camels while 2% cases of
33 clinical mastitis were recorded. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus*
34 *dysgalactiae*, *Bacillus cereus* and *Corynebacterium kutscheri* were isolated with characteristic
35 beta and alpha hemolysis patterns. Chi-square analysis showed significant difference as $p < 0.05$
36 among various species of bacteria ($\chi^2 = 21.649$, P-Value = 0.0001, df = 3). Antibiogram showed
37 Gentamicin, Norfloxacin, Oxytetracycline as most effective therapy for mastitis in camel.

38 **Key words:** *Trypanosoma evansi*; Camel; Hematology; Mastitis, *Anaplasma* spp.

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40

1. INTRODUCTION

41 The Pakistani camel is well known for higher milk production. The Mareecha breed is
42 considered as the best milk producing camel of the world, with an average milk yield of 4179L
43 per year (1). Average lactation length is 270 to 540 days and the total milk production is 1300 to
44 4200L/ lactation. Camel milk contains high amounts of proteins, fat, minerals and C vitamins.
45 The high vitamin C content is important in desert areas where green food is not easily available.
46 Camel milk has higher phosphorus content than any other domestic animal. The fat content in
47 camel milk is equal to cow milk. So, it is clear that camel milk is superior to the milk of other
48 domestic animals (2). In desert areas of Punjab, Pakistan, camel is mainly kept for milk
49 production even when milk from cattle is not available (3). Little research has been done on
50 camel disease especially mastitis as compared to other ruminants in Pakistan. Mastitis in the
51 camels has been reported and its prevalence has increased during the past decade (4). (5). Other
52 frequent bacterial diseases of camel reported by field veterinarians in Punjab, Pakistan are
53 Hemorrhagic septicemia, Black quarter, Tetanus, Brucellosis and Anthrax (5). Among vector-
54 borne protozoan diseases, Trypanosomiasis or Surra, babesiosis, anaplasmosis and
55 trypanosomiasis are principally important (6) and characterized by elevated body temperature,
56 anemia, emaciation, lymphadenopathy, edema and sudden death (7). In Pakistan trypanosomiasis
57 and mastitis are prevalent as a major threat to the camels causing heavy financial losses. After
58 an outbreak of trypanosomiasis during 1985–1986 in camels of Baluchistan, various studies have
59 been directed on camel trypanosomiasis in numerous areas of Pakistan (8). Hematology is an
60 attractively important biomarker in veterinary medicine, worldwide. This biomarker gives us an
61 opportunity to explore the physiological and pathological states of the organism. Blood protozoa
62 mentioned above have hemato-pathological effects in host animals (9). The intensity, duration
63 and cure from animal to animal for mastitis and blood protozoan differ depending upon the risk

64 factors. (10). Mastitis occurs after invasion of bacteria in teat canal and mammary glands. The
65 reported causative agents of mastitis are Streptococci, Staphylococci, Escherichia coli,
66 Corynebacterium, Pseudomonas spp, and mycobacterium tuberculosis (11). There is paucity of
67 information about the etiological agents associated with camel mastitis. Few available literatures
68 indicate that Staphylococcus aureus, Streptococcus spp. (12), Micrococcus spp. Streptococcus
69 agalactiae, coagulase negative staphylococci, Staphylococcus epidermidis, Pasteurella
70 haemolytica, Escherichia coli and Corynebacterium spp. (13) have been implicated as causes of
71 mastitis in camels. Sub-clinical mastitis causes the greatest overall losses in most dairy herds and
72 is 3-40 times more prevalent than the clinical mastitis. In sub clinical mastitis there is a reduction
73 in milk yield, with no obvious clinical signs although milk quality is affected (14). Depending
74 upon severity of mastitis, milk quality is altered not only in nutrient contents but also in increase
75 of somatic cell count, leakage of intracellular contents into the milk, alteration in ionic balance
76 and decrease in milk production. The shelf life of mastitis milk is also reduced. Such milk if used
77 for yogurt and cheese production would result in low quality final products (15).Treatment with
78 a variety of antibiotics and anti-inflammatory drugs can be valuable when there is clinical
79 mastitis. Animal factors (e.g., parity, stage of lactation, somatic cell count history) and the
80 virulence of pathogens involved affects the protocol of treatment, hence, animal-specific
81 treatment of clinical mastitis is often indicated (16). Keeping in view the economic importance
82 of camel and the losses caused by mastitis and blood protozoan infections this study was
83 designed to find causative pathogens for blood protozoan and mastitis infection in one humped
84 camel (*Camelus dromedarius*) and to suggest the appropriate antibiotic therapy in Cholistan,
85 Rahim-Yar-Khan and Dera Ismail Khan districts of South Punjab, Pakistan so that further steps
86 in control and prevention of these economically important diseases may be devised.

87 **2. Materials and Methods:**

88 **2.1.Source of Samples (Inclusive and Exclusive Criteria)**

89 Samples were collected from all three geographical areas of southern Punjab mentioned above.
90 Camels kept on free range grazing and open housing without holding sheds were included in the
91 study. For study on mastitis in camels the lactating camels were included in this study Camels
92 showing signs of fever, anorexia, dullness, depression, pale mucous membranes, anemia, weight
93 loss, facial paralysis, thin hump and dropped to one side and females with history of abortions
94 were suspected for being infected with blood parasites (17).The age of individual animals was
95 documented on the basis of information provided from the owners. The animals were distributed
96 in four age groups. The data regarding animals' clinical history and treatment protocols were
97 also entered in data capture form.

98 **2.2.Collection of samples:**

99 100 lactating camels were examined for clinical and subclinical mastitis,thirty three each
100 from Dera Ghazi khan and Rahim Yar Khan while 34 from Cholistan. Clinical mastitis was
101 diagnosed on the basis of presence of physiological biomarkers of changes in udder (signs of
102 inflammation, differences in texture, disproportionate quarters) and changes in milk (taste, color,
103 consistency, presence of flakes) while the subclinical mastitis was diagnosed by Surf Field
104 Mastitis Test Milk samples (10 ml each) were collected in sterilized, screw capped test tubes
105 and transported to Microbiology Laboratory at University of Veterinary and Animal Sciences,
106 Lahore . . 300 camels, 100 each from different common grazing and watering point of three
107 project sites mentioned above were selected. Out of 300 samples, 150 blood samples were
108 collected from clinically suspected animals as per physiologic biomarkers mentioned before for
109 blood protozoa while 150 samples were collected from apparently healthy animals to compare
110 normal blood parameters. Approximately 5 ml blood sample was collected aseptically by Jugular

111 vein puncture from each camel and transferred into anti-coagulants vacationers containing
112 EDTA as an anticoagulant for hematological examination and PCR (*Trypanosoma evansi*
113 confirmation)

114 **2.3.Blood Smear Examination for blood parasites:**

115 Preparation of blood smear slides was done from the drops of blood collected from jugular vein
116 for parasitological examination. Blood parasites were identified by their morphological
117 characteristics (17). Changes in blood cells morphology were also documented.

118 **2.4.Hematological examination and serum biochemistry**

119 Blood sample for hematological studies were processed at Clinical Medicine Laboratory, UVAS
120 Lahore. Hemoglobin (Hb) estimation, Erythrocytes Sedimentation Rate (ESR) , Total
121 Erythrocytes Count (TEC), Total Leukocytes count (TLC) , Differential Leukocytes Count
122 (DLC) , PCV was done by hematology analyzer. Serum biochemical tests including total serum
123 proteins, serum albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate
124 aminotransferase (AST) and gamma glutamyltransferase (GGT) were done by serum chemistry
125 analyzer. The values obtained were compared with normal documented values (18).

126 **2.5.DNA extraction and PCR for trypanosomiasis:**

127 DNA was extracted from blood samples with Tiangen Genomic DNA extraction kit (Tiangen
128 Biotech, Beijing, China) as recommended by manufacturer. PCR was performed with two *T.*
129 *evansi* specific primer sets that amplified a 228 bp region. *T. evansi* (F) and *T. evansi* (R)
130 primers were used in PCR in a final volume of 20 µL consisting of 2 µL of 10x PCR buffer (50
131 mM Tris-HCl; pH 8.5; 50 mM NaCl), 2 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of each
132 primer, 5 U Taq Polymerase, 2 µL of DNA template (10 ng/µL) and distilled water. The DNA
133 was amplified with 5 min denaturation at 94°C, followed by 35 cycles (denaturation at 94°C for

134 45 sec, annealing at 64.6°C for 1 min and extension at 72°C for 90 sec) and a final extension at
135 72°C for additional 10 min.

136 **2.6.Surf Field Mastitis Test for subclinical Mastitis:**

137 A 3% reagent solution of common detergent “Surf Excel” (Lever Brothers, Pvt. Ltd. Pakistan)
138 was prepared and slime or gel formation was noted for the positive subclinical test.

139 **2.7.BACTERIOLOGICAL EXAMINATION**

140 **2.7.1. Primary isolation of bacteria**

141 Blood agar base, melted and cooled to 45 to 50°C, 5% (vol/vol), was mixed with sterile
142 defibrinated blood by rotation. Each milk sample was streaked on sterile blood agar plates and
143 incubated at 37°C for 24-28 hours. The colonies grown were and examined as recommended by
144 the National Mastitis Council (19).After examination of primary growth smears from different
145 colonies were made and stained with Gram’s staining method followed by examination under the
146 microscope.

147 **2.7.2. Purification and identification of bacteria**

148 The primary growth was purified by frequent sub-culturing on selective and differential media.
149 Mannitol salt agar (Staphylococcus -110 medium) and blood agar was used for purification and
150 differentiation of the primary isolates on the basis of cultural and morphological characteristics,
151 motility, hemolytic and metabolic properties as described in Bergeys identification chart.
152 Cultural character of shape, color, surface edge, structure and consistency of colonies for various
153 isolates were recorded . Staining Techniques used to identify bacteria were Gram Staining (20),
154 Spore Staining (21), Capsule staining (22) and Acid fast staining (23).The shapes and
155 arrangement of the organisms was recorded. Sterilized blood agar was poured into petri plates.

156 These plates were inoculated with each test organism and incubated at 37°C for 24 hours and
157 type of hemolysis (alpha, beta, and gamma) was recorded.

158 **2.7.3. Fermentation reaction / tests:**

159 Glucose, Mannitol, Lactose and Sucrose were used in the tests. The inoculated tubes along with
160 control (un-inoculated) tubes were incubated at 37°C for specified period (24 to 48 hrs). These
161 tubes were observed daily for the presence or absence of acid in tubes and gas formation in
162 Durhams tubes. Change in color from red / pink to yellow indicated acid production.

163 **2.7.4. Biochemical tests:**

164 Biochemical tests were used for the identification and characterization of bacteria Catalase test,
165 Nitrate reduction test, Methyl red test, Voges Proskaur test, Indole Production Test, Citrate
166 utilization test and Urease test.

167 **2.8. Antibiogram:**

168 Sensitivity of each isolated micro-organism against different antibacterial agents was tested on
169 Muller- Hiton agar, supplemented with 5% sheep blood, by disc diffusion method. (24). The
170 antibacterial agents discs used were, Oxytetracycline, Gentamicine, Ampicilin, Amoxacillin,
171 Penicillin, Norfloxacin, Cephradine, Chloramphenicol and Streptomycin. On the basis of
172 diameter of inhibitory zone produced by each antibiotic, the sensitivity was recorded as Resistant
173 (R), Sensitive (S) and Intermediate (I).

174 **3. RESULTS:**

175 Results of the present study on blood parasites showed that out of 300 blood samples 45 were
176 positive for *Trypanosoma evansi* while 15 camels showed Anaplasma infection revealing a total

177 incidence of 15% and 5% for both blood parasites respectively. Out of 150 camels, 20 male and
178 30 female camels were positive for blood parasites. Data analysis regarding sex-wise prevalence
179 revealed that there was no statistically significant difference between male and female animals.
180 Age wise prevalence was 12.5, 17.4, 16.04 and 25.29% in <1 year, 1-2 years, 2-5 years and >5
181 years respectively. The statistical analysis revealed non-significant difference among the
182 different age groups. The results indicated relatively higher incidence in camels of age group > 5
183 years as compared to the other age groups. Results of prevalence regarding sex and age are given
184 in table 2.

185

186 Hematological studies revealed lower values of hematocrit, erythrocyte count and hemoglobin
187 concentration and increased value of erythrocyte sedimentation rate (ESR). Severe neutrophilia,
188 monocytosis, eosinophilia and leukocytosis were also recorded in infected camels as compared
189 to healthy camels. Mean values of albumin and serum total proteins were found significantly
190 reduced in blood parasite affected camels when compared with healthy camels. Serum
191 biochemical tests including alanine aminotransferase, aspartate aminotransferase, gamma
192 glutamyltransferase and alkaline phosphatase also revealed significant difference among healthy
193 and infected animals. A PCR product of 228 bp for *Trypanosoma evansi* was amplified through
194 PCR as shown in Figure 1.

195 The results of study on mastitis showed that out of 100 animals 42 samples were positive for
196 subclinical mastitis on surf field test. Two cases of clinical mastitis were recorded Sample
197 negative for SFMT were 56. Samples were scored Nil, one positive (+), two positive (++) , Three
198 positive (+++) and four positive (++++) according to the severity of thickness of gel formation.
199 The results regarding different risk factors of mastitis like nutrition, body condition, Udder
200 hygiene, Use of teat dips before and after milking indicated that most of the camels investigated
201 during study were underfed with poor body condition. There was decreased trend use of pre and
202 post teat. There was trend of hand washing and udder washing before milking. Milking was
203 mostly done once daily. There was more percentage of subclinical mastitis in when the age was
204 above 10 years. The results were significantly different as $p<0.05$. The results are represented in
205 figures 2, 3 and 4 .

206

207 **3.1.Bacterial Staining:**

208 The results of bacterial staining was obtained performing gram staining and special staining were
209 that *Staphylococcus aureus* was gram positive cocci, appeared in clusters, non spore forming,
210 nonmotile bacteria.*Streptococcus agalactiae* and *Streptococcus dysgalactiae* were gram positive
211 cocci arranged in chains,non motile, non spore forming and non flagellated bacteria.Capsule was
212 absent.*Corynebacterium kutscheri* appeared as pleomorphuc gram positive rods containing
213 metachromatic granules,non spore forming non capsulated bacteria. *Bacillus cereus* was
214 capsulated, spore forming, gram positive rods.

215 **3.2.Hemolysis Patterns:**

216 *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Bacillus*
217 *cereus* showed beta hemolysis with clearing of red blood cells around the colonies.*Bacillus*
218 *cereus* colonies showed double zone beta hemolysis. *Corynebacterium kutscheri* showed alpha
219 hemolysis that was characterized by green zone around the colonies on blood agar. The results of
220 hemolysis pattern of bacterial isolates are summarized in table 3.

221 **3.3.Biochemical Tests:**

222 The Biochemical tests that were used in this study were Catalase test, Oxidase test, Urease test,
223 Indol test, Nitrate reduction test, Citrate Utilization test, Methyl red test and Voges-Proskauer
224 test. *Staphylococcus aureus* was positive for Catalase,Methyl red, Voges Proskauer's and nitrate
225 reduction. *Streptococcus agalactiae* was positive for oxidase test. *Streptococcus dysgalactiae*
226 was positive for oxidase and nitrate reduction. *Corynebacterium kutscheri* was positive for
227 catalase and urease test. *Bacillus cereus* was positive for catalase and Voges Proskauer's test.

228 **3.3.1. Sugar Fermentation Tests:**

229 .. *Staphylococcus aureus* and *Corynebacterium kutscheri* fermented Mannitol while
230 *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Bacillus cereus* showed no

231 fermentation of Mannitol. Lactose was fermented by *Staphylococcus aureus*, *Streptococcus*
232 *agalactiae*, and *Streptococcus dysgalactiae* while *Corynebacterium kutscheri* and *Bacillus*
233 *cereus* showed no fermentation of Lactose. Sucrose was fermented by all the isolates. The results
234 for sugar fermentation tests indicated negative acid and gas production

235 **3.4.Prevalence of Bacteria**

236 The most dominant isolates were *staphylococcus aureus* followed by *streptococcus agalactia*,
237 *streptococcus dysgalactia* , *Corynebacterium kutscheri* and *Bacillus cereus* species. The results
238 analyzed statistically by chi-Square.The value of chi-Square was 21.619' df value was 3 and p
239 value was 0.0001.The results of chi-Square indicates significant difference among species as p-
240 value <0.05(Table 4)

241 **3.5.Antibiogram Sensitivity Testing:**

242 The in vitro susceptibility tests showed that the most effective drugs were Norfloxacin,
243 Gentamycin, Oxytetracycline and against all isolates followed by Ampicillin, Chloramphenicol,
244 Amoxycillin, Streptomycin, Penicillin and Cephradine. *Staphylococcus aureus* was sensitive to
245 Norfloxacin, Gentamycin, Oxytetracycline. It showed intermediate activity against Ampicillin,
246 Amoxicillin, Chloramphenicol, Streptomycin and Cephradine. It showed resistance to Penicillin.
247 *Streptococcus agalactiae* was sensitive to Ampicillin, Chloramphenicol, Gentamycin,
248 Norfloxacin and Oxytetracycline. It showed intermediate activity against Amoxicillin, Penicillin,
249 Cephradine and Streptomycin. The antibiogram for *Streptococcus dysgalactiae* was similar to
250 *Streptococcus agalactiae*. Table 5 shows sensitivity of isolates against the antibiotics. The results
251 of chi-Square indicates significant difference among activity of antimicrobials against different
252 isolates as p- value <0.05.

253

254 **4. DISCUSSION**

255 Infectious diseases and particularly vector-borne protozoan diseases have been serious threat for
256 animal health and productivity in developing countries (25; 26; 27; 28). The overall prevalence
257 of trypanosomiasis and anaplasmosis during present study was 15% and 5% respectively which
258 is in accordance with the previous studies in Pakistan (29). Previously, 13.72% trypanosomes
259 infection was recorded in camels (19). This difference in prevalence could be due to different
260 populations' locations, sample size and prior treatment within the different areas of the country.
261 Present study showed higher prevalence in above 4 years of age as compared less than 3 to 4
262 years of age animals. Previous studies showed 9.67% and 11.25% prevalence respectively (30).
263 Some studies showed significantly low prevalence of 0.7 % in young camels in Pakistan (8). The
264 present study revealed relatively higher prevalence in camels of age group of above 5 years that
265 is also in accordance with the previous studies (31). The relatively higher prevalence of blood
266 protozoan infection in older camels ,5 years of age and above, could be attributed to the poor
267 management, movement from one place to another and heavy stress owing to the involvement of
268 this age group in transportation (32). The results also revealed statistically non-significant
269 difference of infection in male and female animals. These findings are also in accordance to the
270 earlier studies conducted in Cholistan desert (33). Conversely, some studies have reported
271 significantly higher prevalence in female camels (18;8;26) as compared to males while few
272 researchers have also reported higher prevalence in the male camels (34). In short, all sex wise
273 prevalence findings about blood protozoa showed that both sexes are equally susceptible to the
274 disease. However, higher rates of infection in females could be due to the physiological stress
275 leading to decreased resistance and in turn, higher vulnerability to the infection. In contrast,
276 higher infection rates in males can be attributed to the increased physical work leading to fatigue
277 and stress (8). The lower hematological values regarding TEC, PCV and Hb concentrations

278 observed in present study are suggestive of anemia. Anemia has already been reported in blood
279 parasite positive camels and is considered as a major biomarker of these infections in camels
280 (35). The higher value of ESR in infected camels may be due to the anemia and auto-
281 agglutination that is previously detected in this infection (36). Hematological examination in the
282 present study also revealed leukocytosis including neutrophilia, monocytosis, and eosinophilia.
283 Similar results have been previously observed (37). Significantly decreased values of serum total
284 proteins and serum albumin while increased values of ALT, AST, GGT and ALP could be due to
285 centrilobular degeneration as a result of the hypoxia and severe oxidative stress induced by
286 varying severity of blood parasitic infection indicating liver damage. . Similar changes also
287 have been reported in camels due to trypanosomiasis, previously (33). A PCR product of 228 bp
288 for *Trypanosoma evansi* was amplified during the present study that serves as mile stone for
289 vaccine development against trypanosomiasis in further studies.

290 Regarding mastitis the results demonstrated that camels above 9 years were most susceptible for
291 clinical condition that is due to high milk production at this age, with low numbers of somatic
292 cell counts. . The unhygienic milking and poor management practices in the study area might
293 also have contributed to the high prevalence of mastitis in the camel herds examined during the
294 present study. The Gram-positive cocci were the main pathogens isolated from infected camel
295 milk samples. Many authors also reported that these pathogens are major mastitis causing agents
296 in she-camels with mastitis (13). The results of present study indicate that *Staphylococcus aureus*
297 was the main isolated bacterium (52.3%) in subclinical mastitis of animals indicating the
298 environmental distribution of this organism. Isolation of the etiological agent *Staphylococcus*
299 spp, *Streptococcus* spp and *corynebacterium* from mastitis cases was also previously reported
300 (13) in Pakistan. During the present study *Streptococcus agalactiae* were also the second most
301 common causes of camel mastitis. *Strep. dysgalactiae*, *Corynebacterium* spp and *Bacillus* spp

302 were also isolated in the present study from infected camel milk. These isolated bacteria have
303 also reported to be zoonotic in many reports worldwide. Previous and present reports of the
304 above cited bacteria from camel milk also points to the growing concerns of antimicrobial
305 resistance. The indiscriminate use of unprescribed antimicrobial drugs for disease control in
306 camels is suspected to play a role in the spread and persistence of antimicrobial-resistant
307 zoonotic bacteria. The most effective antibiotics, recorded by antibiogram in present study, were
308 Gentamicin, Norfloxacin, Oxytetracycline . However, previous studies have shown Norfloxacin
309 and Chloramphenicol were the most effective drugs (38).

310 **5. CONCLUSION**

311 From the findings of this study it can be concluded that trypanosomiasis and
312 anaplasmosis are present in desert conditions of Pakistan and cause disparities in various
313 hematological as well as biochemical parameters of camels. The results showed that acaricidal
314 use must be communicated to camel owners in southern Punjab through appropriate extension
315 methods and tools. *Staphylococcus aureus* was dominant cause of sub-clinical mastitis. It was
316 attributed to inadequate hygienic condition of environment. The isolation of genera of pathogenic
317 bacteria from the camel milk samples suggests the need for strict hygienic measures during the
318 production and handling of camel milk to reduce public health hazards. Thus, good management
319 practices with proper sanitation and is required to prevent the incidence of mammary infection in
320 camels in the study areas. Education of the camel owners about the importance of hygienic
321 milking practices would minimize mastitis. It is beneficial if the lactating camels in the herd are
322 screened regularly to diagnose sub-clinical mastitis and to achieve maximum milk production.
323 Correct and good milking techniques are essential in the prevention of both environmental and
324 contagious mastitis. The recommended screening and milking protocol must be implemented.
325 Norfloxacin, Gentamycin, and Oxytetracyclin were most effective drugs against all isolates

326 during the present study but antibiotic policy need to be devised and implemented to avoid
 327 present and future problem of antibiotic resistance in camels of Southern Punjab in Pakistan.

328

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430

Table 1: Interpretation of Samples for SFMT :

CMT score	Interpretation	Visible reaction
-	Negative	Normal
±	Trace	Slight precipitation
+	Weak positive	Precipitation without gel formation
++	Distinct positive	Thick gel formation
+++	Strong positive	Strong gel which is cohesive with a convex surface.

Table 2: Prevalence of blood protozoa in camels in Cholistan desert

Sex/age	No. of camels	Positive		Negative	95% CI	Odd Ratio/ P value
		n	%			
Sex						
Female	140	30	21.43	110	9.20-20.83	OR =0.90 [reciprocal = 1.11]
Male	160	25	15.63	135	10.61-21.88	
Overall	300	55	18.33	245	11.29-19.38	-
Age groups						
Less than 1 year	64	8	12.5	56	4.91-20.44	
1-2 years	68	12	17.64	56	5.62-21.12	
2-5 years	81	13	16.04	68	7.35-22.37	Mantel-Haenszel chi-sq P < 0.053
5 years to onward	87	22	25.29	65	14.10-31.43	
Overall	300	55	18.33	245	11.29-19.38	-

Table 3: Hemolysis Patterns of Bacterial isolates

Bacteria	Hemolysis
<i>Staphylococcus aureus</i>	Beta hemolysis
<i>Streptococcus agalactiae</i>	Beta hemolysis
<i>Streptococcus dysgalactiae</i>	Alpha hemolysis
<i>Corynebacterium kutscheri</i>	Gamma hemolysis
<i>Bacillus cereus</i>	Beta hemolysis

Table 4: Number and Percentage of Bacterial Isolates from samples positive for**Surf Field Mastitis Test (SFMT)**

Bacterial isolate	Number	Percentage
<i>Staphylococcus aureus</i>	22	52.3
<i>Streptococcus agalactia</i>	9	21.4
<i>Streptococcus dysgalactia</i>	7	16.6
<i>Corynebacterium kutscheri</i>	3	7.1
<i>Bacillus cereus</i>	1	2.3

Chi-square analysis showed significant difference as p<0.05 among various species of bacteria ($\chi^2=21.649$, P-Value= 0.0001, df=3).

Table: 5 : Antibiogram Sensitivity of bacterial isolates

Bacterial species	Identified Sample Number	Antibiotic Disc								
		Am	Amx	Chl	Gen	OTC	Pen	Str	Cep	Nor
<i>Staphylococcus aureus</i>	1	I	I	I	S	S	R	I	I	S
	2	I	R	I	S	R	R	I	R	S
	3	I	R	I	S	I	R	I	I	S
	4	I	R	I	I	I	R	I	I	S
	5	I	R	I	I	I	R	I	R	S
<i>Strertococcus agalactiae</i>	1	I	I	S	S	S	I	I	I	S
	2	I	I	S	S	S	I	I	I	S
	3	I	I	I	S	I	R	I	R	S
	4	I	I	I	S	S	R	I	I	S
	5	I	I	I	S	I	R	I	I	S
<i>Strertococcus dysgalactiae</i>	1	I	I	I	S	I	I	I	I	S
	2	I	I	I	S	S	R	I	I	S
	3	I	I	S	S	I	R	I	I	S

	4	I	I	I	S	I	R	I	I	I
	5	I	I	I	S	I	I	I	I	I

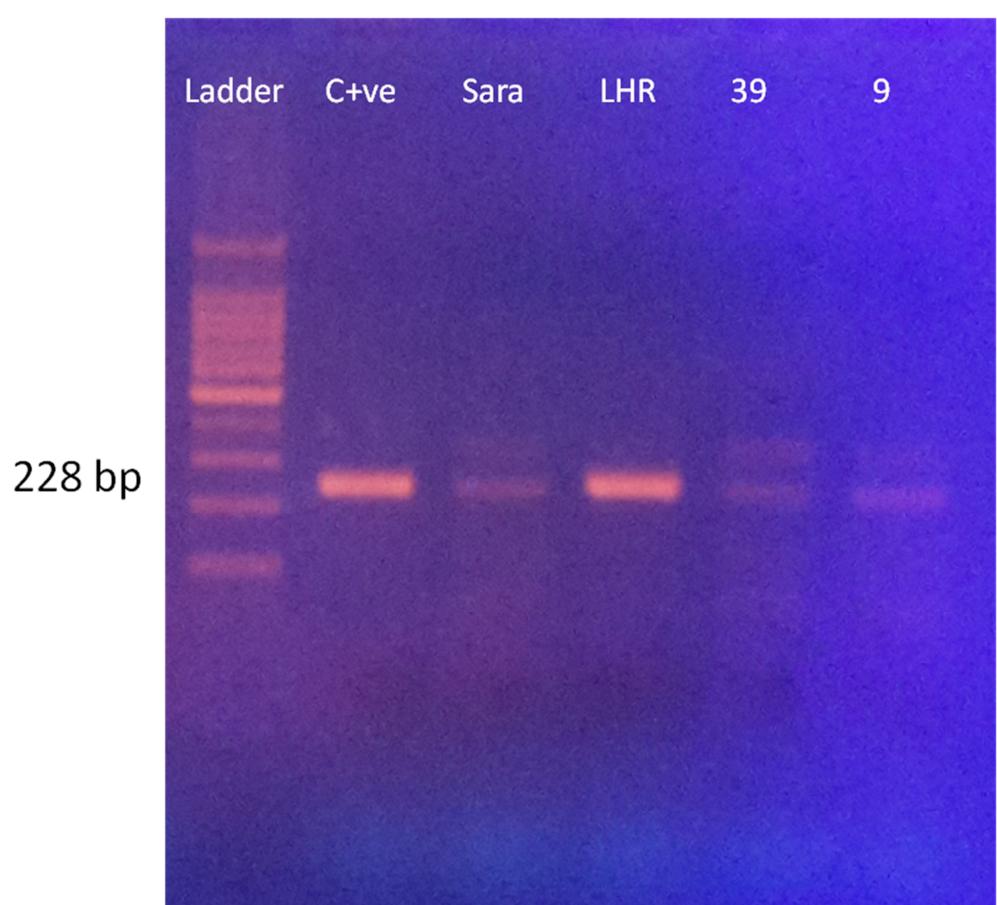
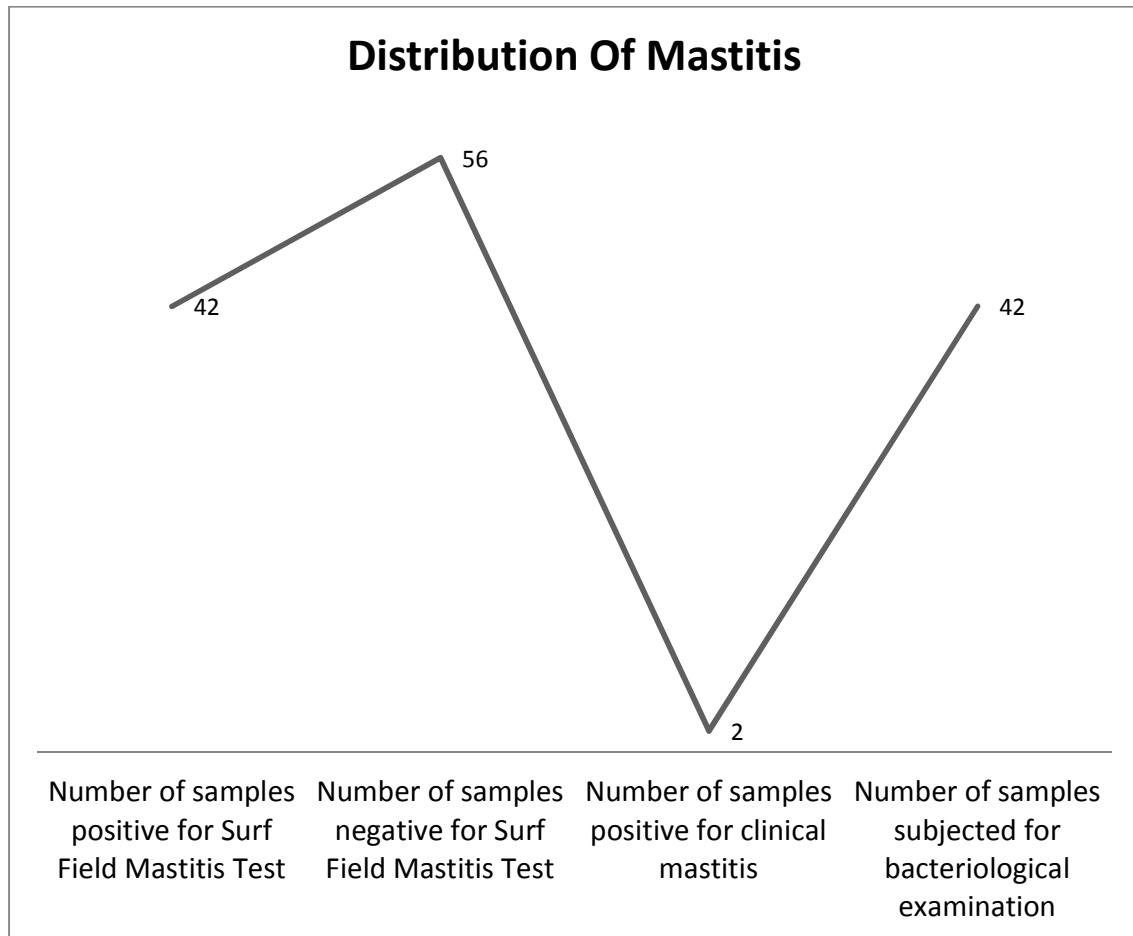
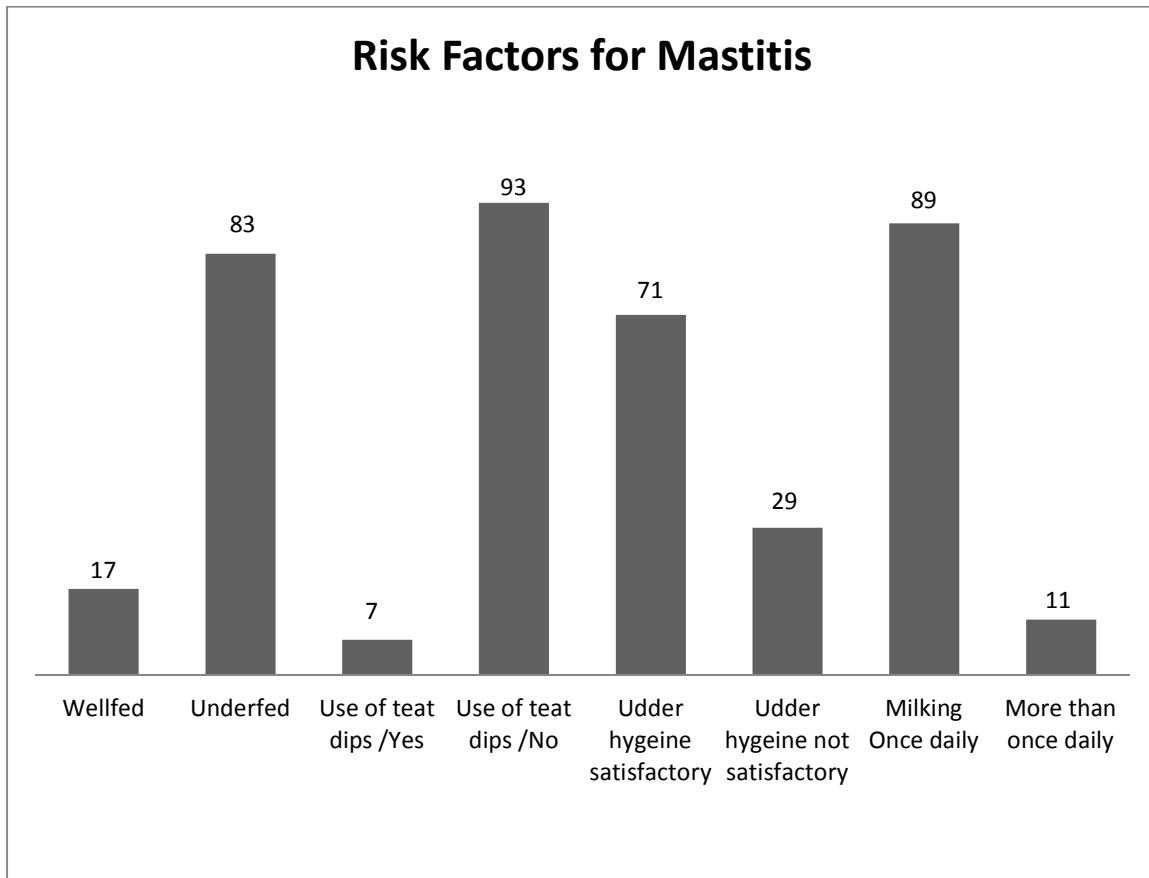


Figure 1: PCR result showing 228 bp DNA amplicon



Chi-square analysis showed significant difference as $p<0.05$ among various species of bacteria ($\chi^2=47.120$, $P\text{-Value}= 0.000$, $df=2$)

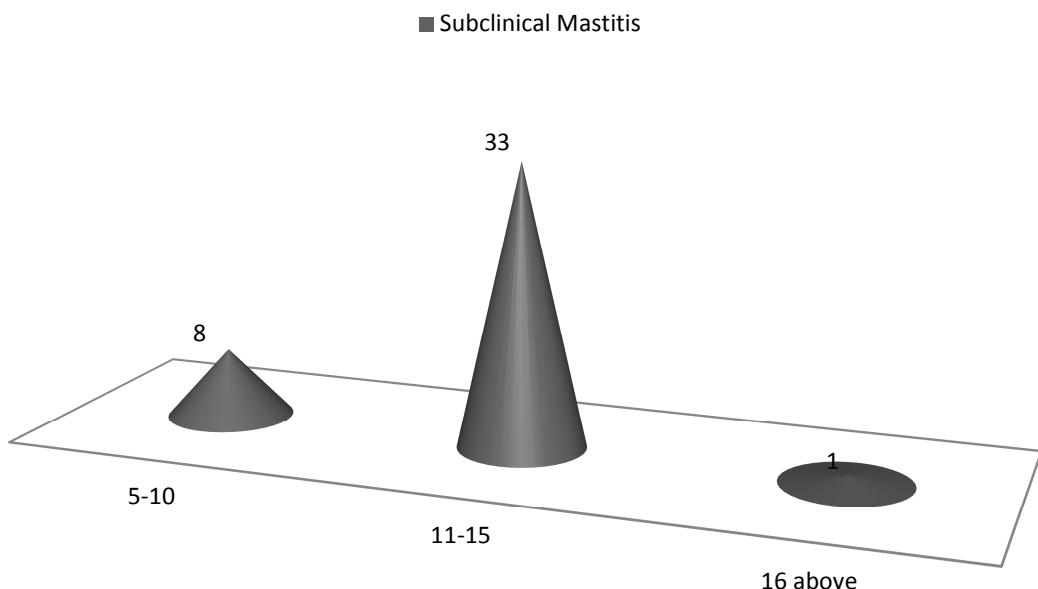
Fig 2 Distribution of mastitis in milk samples of camel (n=100)



Chi-square analysis showed significant difference as $p<0.05$ for nutrition , use of teat dips, udder hygiene and milking practices. ($\chi^2=57.450$, $\chi^2=17.640$, $\chi^2=73.960$, $\chi^2=60.840$ $P=0.000$ and $df=1$ respectively).

Fig 3 Risk Factors of Mastitis in Camels Regarding Management

Distribution of Subclinical Mastitis according to Age



Chi-square analysis showed significant difference as $p<0.05$ ($\chi^2=13.714$, $p=0.0001$.df=1).

Fig 4: Distribution of Subclinical mastitis in camels according to Age

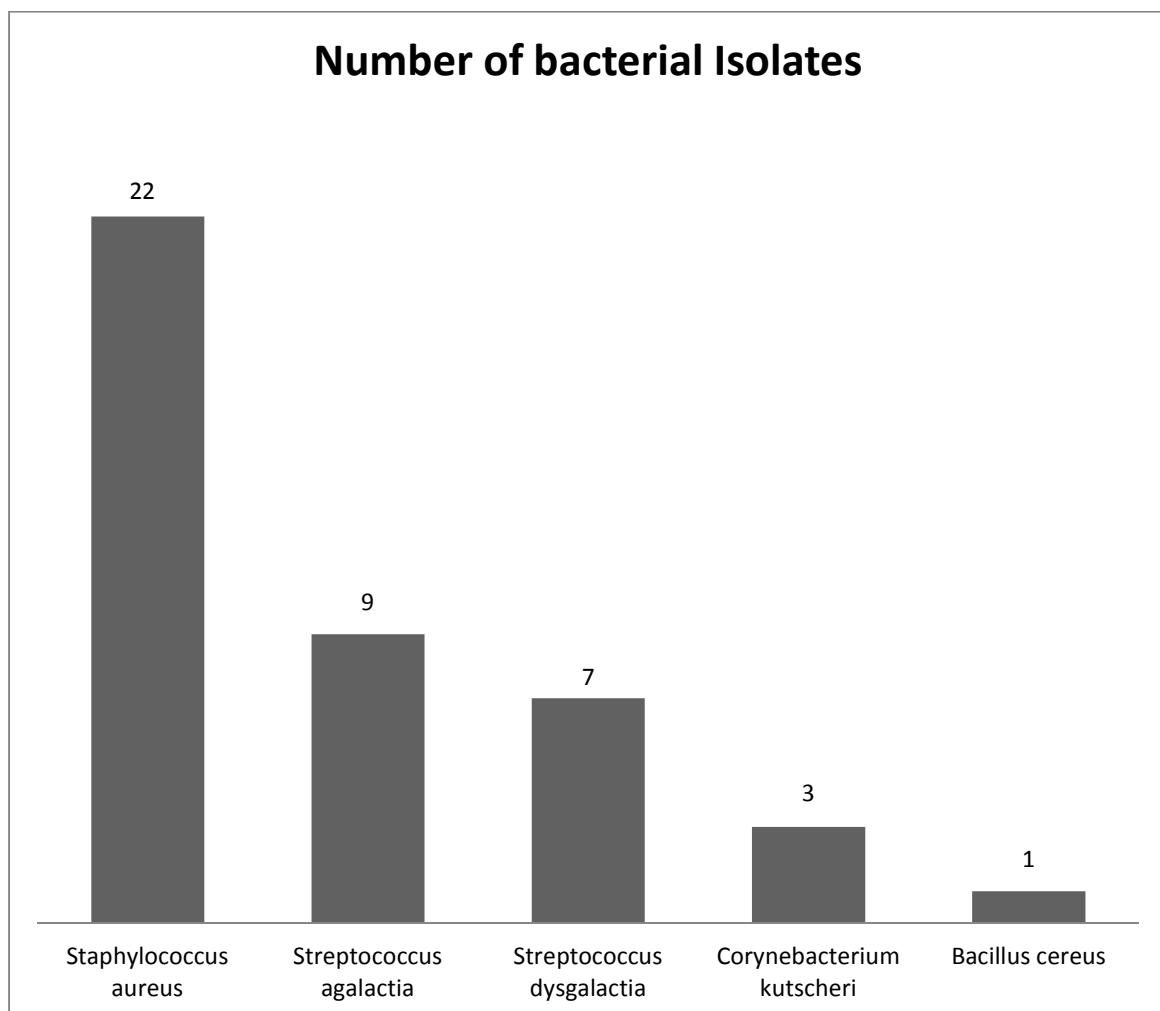


Fig 4 Number of bacterial isolates in SFMT positive Milk samples