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Mastitis and Associated Histo-pathological Consequences in the Context of Udder Morphology

RIAZ HUSSAIN‡, M. TARIQ JAVED, AHRAR KHAN[1], FAZAL MAHMOOD AND RAZIA KAUSAR†
Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
†*Department of Anatomy, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan*
‡*University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan*
[1]Corresponding author's e-mail: ahrar1122@yahoo.com

ABSTRACT

This study determined association of some morphometric characteristics of udder with mastitis in dairy cattle. For this purpose; 100 animals each negative and positive for mastitis by California Mastitis Test and bacteriology, were selected from amongst those brought for slaughter at Faisalabad abattoir. Morphometric observations on udder were recorded pre-slaughter; whereas, samples from mammary tissues were collected post-slaughter for histopathology, alkaline phosphatase and protein activity. *Staphylococci* were predominant (95% C.I.=31.7–50.8) being significantly higher than *Streptococci* (95% C.I.=9.8–24.2) and *Escherichia coli* (95% C.I.= 3.8–14.6) in mastitic cattle. Morphometric comparisons revealed lower (P<0.0001) teat and teat canal length; whereas, higher (P<0.0001) teat base and mid diameter in mastitic cattle. The results of univariate and bivariate logistic regression including species and individual variable in the model revealed association of teat involved, teat shape, udder shape, somatic cell count and teat/udder pathology with the occurrence of mastitis. Histopathological findings revealed significantly lower alveolar diameter, number of alveoli and alveolar epithelial cell population in mastitic dairy cattle. Mammary tissues from mastitic animals also demonstrated weak alkaline phosphatase and protein activity. Results of the study may have application in selection of dairy animals and in a better understanding of the pathological consequences of mastitis.

Key Words: Cattle; Mammary gland; Mastitis; Bacteriology; Pathology; Histochemistry

INTRODUCTION

In Pakistan, about 30–35 million people are involved in rearing cattle and buffaloes (Government of Pakistan, 2007–2008). On the basis of milk production (43.562 million tons), Pakistan is ranked at third in the world in relation to milk production (Afzal, 2010). Mastitis, inflammation of mammary parenchyma, in early lactation results in long term production loss and risks of premature culling from the herd. Mammary gland infection may result in release harmful toxins in the udder (Yousaf *et al*., 2010) and lesions may vary from increased milk leukocytes counts with no gross changes in milk to increased vascular permeability (Oviedo-Boyso *et al.*, 2007; Ibrahim *et al.*, 2011) or develop fibrosis or severe toxemia (Costa, 1998). Milk leukocytes/somatic cells consist of several types, including neutrophils, macrophages, lymphocytes and a small percentage of epithelial cells (Abera *et al.*, 2010). The activities of the resident and newly recruited leukocytes during the early stages of mastitis play a vital role in the establishment of intra-mammary infection. Marked leukocyte infiltration during mastitis is detrimental to the developing mammary parenchymal tissue (Nickerson, 2009) ultimately leading to milk loss (Piepers *et al.*, 2009).

Milk protein synthesis in mammary tissue is a complex mechanism under the influence of local and systemic hormones along with some other factors those affect milk yield (Silanikove *et al.*, 2006; Khaliq & Rahman, 2010; Hussain *et al*., 2010). In lactating animals, mammary gland is characterized by major changes including increase in number of alveoli, alveolar lumen and decrease in connective tissue (Holland & Holland, 2005; Patel *et al*., 2007). Characterization of mammary parenchyma indicates the impact of potential milk production on development and secretory cell differentiation (Capuco & Akers, 1999). Different potential measures to decrease mastitis include selection of animals which are less prone to develop infection, culling of more susceptible animals, potential effects of teat-end size, morphology of teat/udder and association of different lesions (Slettbakk *et al*., 1995; Bhuto *et al*., 2010). This paper describes association of some morphological traits of udder with mastitis in dairy cattle in addition to the bacteria involved; and histopathology, alkaline phosphatase and protein activity in mammary tissues.

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MATERIALS AND METHODS

Milk examination: A total of 100 each mastitis positive and negative (California mastitis test; Schalm *et al.*, 1971) animals were randomly selected for this study from amongst the dairy cattle brought for slaughtered at Faisalabad (Pakistan) abattoir. The somatic cell and differential leukocyte counts in milk were carried out as described by Lindmark-Månsson *et al.* (2006). For bacterial isolation, standard morphological and biochemical tests were carried out (National Mastitis Council, 1999). Isolates of different bacteria were biotyped using commercially available kits (API, BioMerieux, France).

Macroscopic examination and biometry of udder: The mammary glands of all the animals were analyzed for presence of any gross lesions. The udder configuration and teat/udder pathology was recorded following Bhutto *et al.* (2010). Teat and streak canal length and teat diameter from apex, mid and base of all the teats were measured using a vernier caliper after the animals were slaughtered. The teat and udder shape was documented following Shukla *et al.* (1997).

Histopathological and Histochemical Investigations: At slaughter, the udder was incised quickly and mammary tissue parenchyma were immediately fixed in Bouin's solution for 24 h and then in 70% ethanol. For histological observations, 3 to 4 µm thick sections were deparaffinised, hydrated and stained with acid hematoxylin and eosin (Bancroft & Gamble, 2008). For demonstration of alkaline phosphatase, protein expression and alveolar cell population, 4 µm thick sections from mammary parenchyma embedded in paraffin were cut and dipped in xylene for 10 min to remove paraffin and cleared in different series of ethyl alcohol (100, 90, 70 & 50%) for 3–4 min in each grade. Afterward, tissue sections were washed in running tap water for 2 to 3 min. Alkaline phosphatase activity (Elsayed *et al.*, 2009), protein loci (mercury–bromophenol blue techniques; Elsayed *et al.*, 2009) and alveolar cell population in mammary glands (Elsayed *et al.*, 2009) were also determined.

Statistical analysis: The data were analyzed using Chi-square and logistic regression analysis, odd ratio and 95% confidence interval. For other data, general linear model procedure was used for analysis and means were compared by DMR test.

RESULTS

Micro-organism and some udder traits: From slaughtered mastitic cattle, *Staphylococci*, *Streptococci* and *E. coli* alone were isolated from 41, 16 and 8% cases. Prevalence of *Staphylococci+Streptococci*, *Staphylococci+E. coli*, *Staphylococci+Streptococci+E. coli* and *Streptococci+E. coli* was recorded in 15, 13, 4 and 3% in slaughtered cattle. Significantly higher prevalence of *Staphylococci* (C.I. 31.7–50.8) and *Streptococci* (C.I. 9.8–24.2) were recorded in mastitic cattle (Table I).

The results of teat length, teat diameter (apex, middle, and base) and streak canal length in cattle are presented in Table II. The results revealed lower ($P<0.0001$) teat and teat canal length; whereas, higher ($P<0.0001$) teat base and mid diameter were recorded in mastitic cattle.

The bivariate frequency analysis did not show statistical difference for teat shape and teat lesions. However, udder shape ($P<0.0001$), udder position ($P<0.001$) and breed ($P<0.0001$) showed significant difference between mastitic and healthy animals. The mastitis was significantly higher in non-descript than Sahiwal and cross-bred cattle. Similarly, the mastitis was significantly higher in animals having bowl or round shaped than cup shaped udder and in cattle having pendulous udder (Table III). The results of univariate and bivariate logistic regression including species and individual variable in the model also confirmed the association of teat involved, teat shape, udder shape, somatic cell count and teat/udder pathology with the occurrence of mastitis (Table IV).

Histopathological Investigations: The total milk somatic cell count was significantly ($P<0.0001$) higher in mastitic than healthy animals (Table V). Neutrophil population was also significantly ($P<0.0001$) higher in mastitic animals, while macrophages and lymphocytes count was significantly ($P<0.0001$) lower in mastitic cattle. The results of alveolar diameter, number of alveoli and alveolar epithelial cell population varied significantly between mastitic and healthy cattle (Table V). The values for these parameters were lower in mastitic animals than health animals.

Histopathologically, the udder section from healthy cattle showed no pathological lesions and the milk secretion was observed in alveoli (Fig. 1A). However, the tissue sections from mastitic animals revealed mild, moderate or severe atrophy of alveoli (Fig. 1B). The cellular exudate in udder tissue was present in the lumen of the alveoli in varying amounts in a number of cases. The acute inflammatory changes were recorded in 56% and chronic inflammation in 44% cattle. Infected mammary parenchymal tissues showed destruction of alveoli and fibrous tissue proliferation (Fig. 1B). Cellular infiltration mainly was observed in different areas of mammary tissues such as in teat cistern lining, gland cistern and deep parenchyma. Mastitic udder showed significantly lower alveolar epithelial cell population, alveolar luminal diameter and number of alveoli per plate.

Histochemical studies: Tissue sections taken from mammary glands infected with mastitis indicated obvious differences. The mammary glands of non-mastitic animals were more developed and their secretory activity was also higher. The activity of alkaline phosphatase in tissues sections of healthy cattle was observed on the outer boundary of alveolar secretary cells (Fig. 2A). However, tissue sections of the mastitic animals showed weak alkaline phosphatase activity on the outer membrane (Fig. 2B).

Table I: Prevalence of different pathogens recovered from mastitic cattle

Micro-organism	Number of isolate	Positive percentage	95% C.I.
Staphylococci	41	41	31.7 - 50.8
Streptococci	16	16	9.8 - 24.2
Escherichia coli	8	8	3.8 - 14.6
Staphylococci + Streptococci	15	15	9.0 - 23.0
Staphylococci + E. coli	13	13	7.4 - 20.7
Streptococci + E. coli	3	3	0.8 – 8.0
Staphylococci+Streptococci+E. coli	4	4	1.3 - 9.4

Table II: Measurements of various tissues of healthy and mastitic cattle

Parameter/Quarter	Healthy	Mastitic
Teat Length (cm)		
Right Rear	4.77±0.50[a]	4.29±0.49[b]
Left Rear	4.86±0.48[a]	4.44±0.48[b]
Right Front	4.87±0.60[a]	4.40±0.50[b]
Left Front	4.76±0.50[a]	4.44±0.57[b]
Teat Apex Diameter (cm)		
Right Rear	0.69±0.10	0.71±0.60
Left Rear	0.67±0.07	0.70±0.12
Right Front	0.65±0.06	0.69±0.12
Left Front	0.70±0.12	0.73±0.03
Mid Teat Diameter (cm)		
Right Rear	1.87±0.17	1.90±0.28
Left Rear	1.84±0.16	1.85±0.21
Right Front	1.88±0.20	1.89±0.25
Left Front	1.86±0.16	1.89±0.29
Teat Base Diameter (cm)		
Right Rear	2.15±0.23[a]	2.62±0.46[b]
Left Rear	2.15±0.25[a]	2.39±0.11[b]
Right Front	2.07±0.28[a]	2.42±0.22[b]
Left Front	2.31±0.23[a]	2.40±0.09[b]
Right Rear	3.01±0.29[a]	2.60±0.23[b]
Left Rear	2.97±0.24[a]	2.59±0.18[b]
Right Front	3.00±0.29[a]	2.62±0.23[b]
Left Front	2.93±0.29[a]	2.59±0.20[b]

Values (Mean±SD) bearing different superscript in a row differ significantly ($p < 0.001$)

The high (Fig. 3A) and low (Fig. 3B) protein staining density in tissues sections, respectively was observed in healthy and mastitic cattle.

DISCUSSION

Bacteria involved in mastitis have been considered of great significance in the epidemiology of mammary gland disease (Waller *et al.*, 2009). In present study, *Staphylococci* and *Streptococci* were the major pathogens recovered from milk/mammary tissue of slaughtered dairy cattle; whereas, *E. coli* was less frequent. These results are comparable to those reported earlier (Tempelmans Plat-Sinnige *et al.*, 2009). Higher prevalence of *Staphylococci* and *Streptococci* may be attributed to their routine presence on various body parts and survival in teat and skin lesions. Previously, it has been reported that *S. aureus* and *S. agalactiae* show a rapid spread and, therefore, these pathogens cause mastitis in a high number of dairy animals (Karahan *et al.*, 2011; Nazifi *et al.*, 2011).

Table III: Bivariate frequency analysis of different parameters in slaughtered cattle

Parameter	Positive		Negative	Mantel-Haenszel Chi
	n	%		
Teat shape				
Pointed	19	39.58	29	>0.6890
Cylindrical	37	56.06	29	
Round	27	58.70	19	
Flat	17	42.50	23	
Teat lesions				
None	36	50.70	35	<0.0192
Teat apex injury	15	48.39	16	
Skin abrasion	9	26.47	25	
Inflammation	12	63.16	7	
Cord formation	9	64.29	5	
Haemorrhages	6	75.00	2	
Necrosis	5	45.45	6	
Udder edema	8	66.67	4	
Breed				
None descript	73	62.39	44	<0.0001
Cross bred	21	30.88	47	
Sahiwal	6	40.0	9	
Udder shape				
Cup	20	25.97	57	<0.0001
Round	52	65.00	28	
Bowl	28	65.12	15	
Udder position				
Non pendulous	49	40.16	73	<0.0005
Pendulous	51	65.38	27	

Table IV: Logistic regression procedure for various parameters showing significant association in cattle

Variable	Odd ratio	95% confidence limits		P-value
		Lower	Upper	
Univariate logistic regression and individual variable in the model				
Teat involved	0.067	0.034	0.130	<0.0001
Teat shape	0.775	0.642	0.935	<0.01
Teat lesions	0.923	0.843	1.011	<0.05
Udder shape	0.457	0.348	0.600	<0.0001
Bivariate logistic regression including species and individual variable in the model				
Teat involved	0.067	0.034	0.130	<0.0001
Teat shape	0.772	0.639	0.932	<0.01
Udder shape	0.456	0.347	0.599	<0.0001
Somatic cell count	0.392	0.160	0.961	<0.05

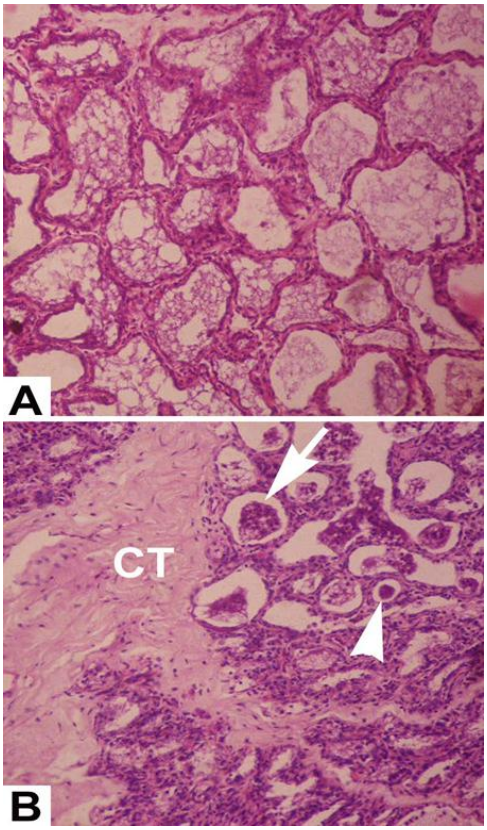
In present study, the prevalence of mastitis was higher ($P<0.0001$) in quarters with small teat and streak canal length and large teat diameter. It could be due to the reason that the pathogens have to travel less distance to establish infection in mammary glands. Association of these factors with occurrence of mastitis has already been established worldwide (Chrystal *et al.*, 1999; Klaas *et al.*, 2004; Bhutto *et al.*, 2010). The frequency analysis showed higher prevalence of mastitis in cattle having pendulous, round and bowl shaped udder. Previously, higher prevalence of mastitis in impaired teat and long udder shape has been reported (Klaas *et al.*, 2004; Bhutto *et al.*, 2010). The logistic procedure also confirmed that teat lesions, teat/udder shape and pendulous udder were significantly associated with mastitis. The association of pendulous udder with teat/udder injuries has also been reported previously

Table V: Milk leukocyte count and histo-morphometry of mammary parenchyma in cattle

Parameter	Healthy	Mastitic
Milk somatic cell counts		
Total cell count ($x10^5$)	3.70±0.49[a]	56.06±22.56[b]
Neutrophil (%)	23.16± 3.08[a]	57.21± 4.35[b]
Macrophages (%)	27.25±3.82[a]	22.63±3.38[b]
Lymphocyte (%)	30.35±3.58[a]	12.73±1.71[b]
Alveolar diameter (µm)		
Long diameter	106.65±13.08[a]	71.92±11.64[b]
Short diameter	71.24±12.86[a]	44.33±7.22[b]
Minimum long diameter	75.28±15.25[a]	54.13±6.15[b]
Maximum long diameter	115.70±15.08	91.18±21.06
Minimum short diameter	61.12±14.61[a]	32.61±5.73[b]
Maximum short diameter	80.43±18.88[a]	56.96±13.63[b]
No. of alveoli/plate	87.57±7.33[a]	59.01±6.71[b]
Alveolar cell number	43.50±3.22[a]	10.35±2.73[b]

Values (Mean±SD) bearing different superscript in a row differ significantly ($p < 0.0001$)

Fig. 1: Photomicrograph of mammary tissue from healthy cattle (A) showing alveoli containing milk secretions. In part B, mammary tissue from mastitic cattle showing proliferation of connective tissue (CT), severe cellular exudate (arrow) and atrophy of alveoli (arrow head). H and E Stain, 200X



(Shukla *et al.*, 1997; Breen *et al.*, 2009; Bhutto *et al.*, 2010).

The most common histopathological findings in this study were severe cellular infiltration, atrophy of alveoli, broken alveoli, increased stromal tissue and presence of

Fig. 2: Photomicrograph of mammary tissue from healthy cattle (A) showing increased alkaline phosphatase activity (arrows). In part B, mammary tissue from mastitic cattle showing no alkaline phosphatase activity, 400X

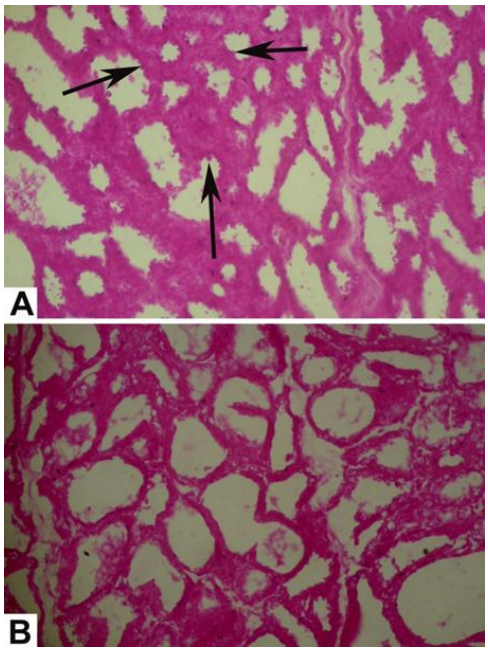
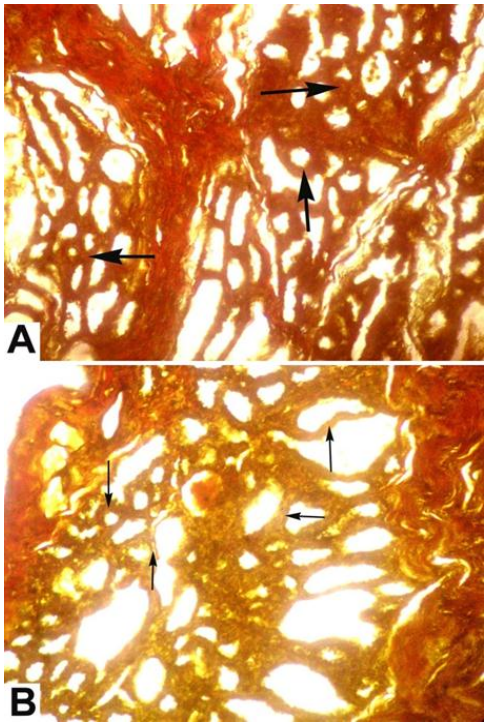


Fig. 3: Photomicrograph of mammary tissue from healthy cattle (A) showing high protein staining (arrows). In part B, mammary tissue from mastitic cattle showing weak protein staining (arrows), 400X



lymphoid nodules in alveoli. The histopathological changes including number of alveoli, alveolar diameter and secretory alveolar cell population significantly decreased in mastitic cattle. These results indicated pathological changes occurring in udder tissue and could be due to severe tissue damage due to different mastitis pathogens. In accessible literature, no reports were found about number of alveoli and alveolar cell population in naturally mastitic animals.

Tissue sections of mammary glands of mastitic cattle revealed low, negligible or altogether no activity (staining) of alkaline phosphatase on the outer membrane of alveolar cells. The negligible to no activity of localization of alkaline phosphatase in mastitic tissues may be related with deactivation of this enzyme owing to negative regulatory process of mammary gland. Alkaline phosphatase, a membrane-associated glycoprotein enzyme, increases hydrolysis of phosphates and is located mainly on the outer cellular membranes of tissues having vigorous transport processes (Murray & Ewen, 1992). Previously, in mastitic cattle no reports about the localization of alkaline phosphatase enzymes have been reported. The weak activity of alkaline phosphatase enzyme could be due to impaired milk secretory mechanism (Hassan, 2004; Silanikove, 2008).

The weak to negligible activity of protein in tissue sections of mastitic cattle in present study could be due to degenerative changes present in the parenchymatous cells, atrophy of lactiferous acini along with connective tissue proliferation and impaired activity of endoplasmic reticulum. No reports are available about the density of protein staining in mastitic cattle. However, different workers reported that protein staining was decreased in advanced stages of lactation (Hassan, 2004; Elsayed *et al.*, 2009). The weak enzyme activity, low protein expression and lower alveolar secretary cell population in udder during involutionary stage have been reported (Elsayed *et al.*, 2009).

In conclusion, *Staphylococci*, *Streptococci* and *E. coli* are the major microorganisms of mastitis in cattle in the area of study. Cattle having lower teat and teat canal length, higher teat base diameter and cattle with bowl or round and lower position are more prone to mastitis than cup shaped or pendulous udder. Histochemical data showed weak alkaline phosphate activity and low protein staining density in tissues sections of mastitic cattle. These findings may be implicated in the selection of dairy animals and better understanding of the pathological consequences of mastitis.

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