



RESEARCH ARTICLE

Possible Risk Factors Associated with Mastitis in Indigenous Cattle in Punjab, Pakistan

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ABSTRACT

The present study was conducted on 453 lactating cattle of various breed present at 21 farms. The milk samples from these animals were collected to diagnose mastitis using California Mastitis Test (CMT). The epidemiological data related to animals and management was collected and analyzed to draw conclusions. The results of t-test revealed significant association between body weight, udder depth, and lower teat end to floor distance ($P < 0.01$) with mastitis. The bivariate frequency analysis revealed significant association for lactation stage ($P < 0.0001$), teat end to floor distance, parity, udder shape, teat shape, live body weight, teat and/or udder pathology, use of oxytocin, feeding system and milk leakage with mastitis. The results of logistic regression analysis revealed significant negative association between teat length, frequency of culling and number of attendants, while positive association between mastitis teat involved, teat diameter (apex, mid and base), milk leakage, udder shape, pendulous udder, feeding system, udder depth, teat shape, calf suckling, milk yield, teat and/or udder pathology and live body weight. From the results of present it can be concluded that some risk factors were strongly associated with mastitis in cattle.

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INTRODUCTION

The productive efficiency of dairy herds is negatively affected by poor management, different diseases and various host/environmental risk factors. In modern dairy industry, animals face stress during different physiological phases during development, pregnancy/calving and lactation period. The optimum physiological condition of animal is crucial for the repair/regeneration of damaged/diseased tissues and for optimum functioning of immune system. Physiological stress of any kind reduces the ability of the immune system and when compromised, it favors the occurrence of various diseases including mastitis.

Mastitis occurs if the pathogens gain entry through teat orifice into the mammary tissue. The morphology of teat/udder and the presence of injuries play crucial role in the pathogenesis of mastitis (Chrystal *et al.*, 2001; Ali *et al.*, 2011; Hussain *et al.*, 2012a). Few studies have shown presence of different teat and udder lesions as important risk factors for mastitis (Bhutto *et al.*, 2010) along with inverted and less pointed teat shapes (Slettback *et al.*, 1995). The risk factors including milking techniques, husbandry practices and irrational use of antibiotics to

treat mastitis also favor the occurrence of mastitis (Bhutto *et al.*, 2010). Several studies under varying conditions have identified various risk factors including increased numbers of cows at the premises, milk leakage from teat orifice, heavy milk production, asymmetric udders and use of post-milking teat disinfectants (Barnouin *et al.*, 2005; Breen *et al.*, 2009; van den Borne *et al.*, 2011). There have been few reports published showing association of various risk factors with mastitis in cattle in Pakistan, however, previously none of the published data under local conditions have been analyzed by logistic regression procedures. Thus, there was a strong need to investigate various potential risk factors associated with mastitis involving old but well established dairy farms and those at under private control those are newly established. Therefore, the present study was designed to investigate the possible association of various risk factors in cattle with use of logistic analysis and chi-square methods.

MATERIALS AND METHODS

The study was carried out on a total of 453 cattle present at 21 dairy herds. All the lactating animals at these

farms were tested with California Mastitis Test following the standard protocol (Schalm *et al.*, 1971). The results of the test of each animal were recorded on a specially designed proforma. All other information about each animal was also recorded including the age of the animal, body weight, lactation staged, breed, parity, milk yield, calf suckling, feeding system, use of oxytocin, housing system/space, frequency of culling and number of attendants at the farm. Other parameters including teat length, udder depth, pendulous udder, udder shape, treat shape, teat diameter (apex, middle and base), teat end to floor distance, tail length, teat lesions/injuries and milk leakage from the teat sphincter were also recorded. The teat length and teat diameter were recorded with the help of Vernier Caliper, other all measurements were made with the help of measuring tape.

Statistical analysis: The data obtained in this study were analysed by using SAS 9.1 statistical software (SAS, 2004). Data on teat length and diameter were analyzed by analysis of variance technique and means were compared by t-test. Data on various epidemiological factors were analyzed by using chi-square and logistic analysis procedures. In logistic procedure, the analysis for various risk factors was controlled for farm; farm and breed; farm, breed and age; breed and age. The univariate logistic analysis was also carried out by taking single variable in the model with mastitis as outcome variable. Where appropriate odds ratio and 95% confidence interval were also worked out.

RESULTS

The results for various physical parameters of mastitic and healthy cattle are presented in Table 1. The data analysis by t-test did not show significant difference for parameters including age, parity and tail length between mastitic and healthy cattle (Table 1). However, live body weight ($P<0.01$), teat floor distance ($P<0.01$) and udder depth ($P<0.01$) showed significant difference between mastitic and healthy cattle (Table 1). The live body weight of mastitic cattle was higher, teat floor distance lower and the udder depth was higher than healthy cattle.

Table 1: Analysis of various physical parameters (Mean±SD) of infected and healthy cattle

Parameters	Healthy	Infected/Mastitic	P-Value
Age	6.7±2.3	7.0±2.2	<0.29
Parity	3.2±2.0	3.5±1.9	<0.12
Tail size	36.3±5.0	37.2±5.0	<0.15
Live weight	353.8±57.7	403.2±52.5	<0.001
Teat floor distance	21.3±2.7	17.3±2.3	<0.001
Udder depth	5.5±0.5	7.30±0.7	<0.001

The frequency analysis did not show statistical difference between mastitic and healthy animals for parameters including age, tail length, breed and housing space. However, lactation stage, floor distance, parity, udder shape, teat shape, live body weight, teat and/or udder pathology, use of oxytocin, feeding system and milk leakage ($P<0.005$) showed significant difference between mastitic and healthy animals (Table 2). The

mastitis was significantly higher in animals at their third lactation stage, increase in parity, heavy live body weight, having bowl or round udder, cylindrical, round or flat teats, cattle having teat and udder lesions, animals in which oxytocin was frequently injected for milk letdown, animals fed on stall + grazing ($P<0.01$) and animals having milk leakage ($P<0.005$) throughout the day. However the frequency analysis failed to show significant difference between mastitic and healthy animals for parameters including breed and housing space (Table 2). The results of bivariate logistic regression analysis revealed significant negative association of mastitis with teat length, frequency of culling and teat to floor distance, while positive association with teat involved, teat diameter (apex, mid and base), milk leakage, udder shape, pendulous udder, feeding system, udder depth, teat shape, calf suckling, milk yield, teat lesions and live body weight (Table 3). The multivariate logistic analysis after controlling for farm revealed significant association of all the parameters as were found with bivariate analysis with exception of base diameter of teat, however, an additional association was found with number of attendants at the farm. The multivariate logistic analysis after controlling for farm and breed, breed and age and for farm, breed and age revealed significant association of all the parameters as were found with bivariate analysis with exception of mid and base diameter of teat and frequency of culling, however, an additional association was found with number of attendants and lactation stage.

DISCUSSION

The control of bovine mastitis depends on the detection and elimination of different host and environmental risks factors associated with mastitis. Many risk factors associated with mastitis can be eliminated by adopting hygienic measures, good management and selecting dairy animals which are less prone to mastitis (Nash *et al.*, 2003; Hussain *et al.*, 2012a). The study was carried out to find out the association of certain risk factor with mastitis in cattle under local conditions.

During present study, age, parity and tail length did not show association with mastitis while live body weight, teat to floor distance and udder depth showed significant association. In previous studies, an association of mastitis with age (Barkema *et al.*, 1999; Nyman *et al.*, 2007; Hammer *et al.*, 2012), parity (Parker *et al.*, 2007; Nyman *et al.*, 2009; Kavitha *et al.*, 2009; Salem-Bekhit *et al.*, 2010), body condition (Kivaria *et al.*, 2007) teat to floor distance (Waage *et al.*, 2001; Svensson *et al.*, 2006; Compton *et al.*, 2007; Bhutto *et al.*, 2010) have been reported. However, some workers have not found as association of parity (Slettback *et al.* 1995) live weight (Fregonesi and Leaver, 2001) with occurrence of mastitis. There are differences in literature as for involvement of these parameters with mastitis is concerned. We could not find an association of age with mastitis while most others have which is difficult to be explained. It may be due to the breed differences and genetic makeup of the animals in different regions of the world but this has to be proved in future studies. About data on parity, we found significant association with logistic procedure and

Table 2: Bivariate frequency analysis of different parameters in infected and healthy cattle

Parameters	Positive		Negative	Mental-Haenszel Chi
	n	%		
Lactation stage (months)				
1-4	18	12.00	132	P<0.0001
4.1-8	19	11.31	149	
8.1-12	51	37.78	84	
Floor distance (inches)				
14-17	47	51.65	44	P<0.0001
17.1-20	32	27.83	83	
20.1-23	6	6.52	86	
>23	3	1.94	152	
Parity				
1	17	16.50	86	P<0.05
2-3	23	15.54	125	
4-5	10	9.01	101	
6-7	38	41.76	53	
Tail length (inches)				
20-30	20	25.64	58	P>0.12
30-40	48	18.97	205	
>40	20	16.39	102	
Udder shape				
Cup	15	6.88	203	P<0.0001
Round	36	27.69	94	
Bowl	37	35.24	68	
Teat shape				
Pointed	5	2.55	191	P<0.0001
Cylindrical	36	35.64	65	
Round	18	24.32	56	
Flat	29	35.37	53	
Age				
4&<4	9	11.54	69	P>0.21
4.1-6	32	21.62	116	
6.1-8	19	20.65	73	
8.1-10	16	18.82	69	
10.1-12	12	24.00	38	
Live body weight				
250&<250	0	0	10	P<0.0001
2251-300	6	20.0	24	
301-350	13	6.66	183	
351-400	38	29.45	91	
>400	31	35.23	57	
Breed				
Cross-bred	22	21.15	82	P>0.37
Nondescript	26	21.49	95	
Sahiwal	40	17.54	188	
Teat Lesions				
None	41	15.07	231	P<0.0001
Teat apex	11	23.91	35	
Skin abrasion	12	30.77	27	
Inflammation	7	30.43	16	
Cord formation	6	19.35	25	
Hemorrhages	2	10.53	17	
Necrosis	3	21.43	11	
Udder edema	6	66.67	3	
Oxytocin				
Rare	21	11.41	163	P<0.001
Frequent	67	24.91	202	
Feed system				
Stall feeding	28	13.59	178	P<0.01
Stall feeding+grazing	60	24.29	187	
Housing				
More space	19	14.96	108	P>0.13
Less space	69	21.17	257	
Milk Leakage				
Yes	14	37.84	23	P<0.0005
No	74	21.17	342	

frequency procedure but not with means which is in line with most other reports. However, further studies are required to clarify the association of udder depth and tail length with mastitis.

During present study an association of teat length and teat diameter was found. Similarly, other workers also

Table 3: Logistic regression procedure for various parameters showing significant association in cattle

Variable	Odd ratio	95% Confidence limits		P-value
		Lower	Upper	
Bivariate logistic regression in the model				
Teat length	0.021	0.009	0.050	<0.0001
Frequency of culling	0.021	0.003	0.153	<0.0001
Floor distance	0.534	0.466	0.610	<0.0001
Teat involved	>999.999	120.605	>999.999	<0.005
Apex diameter	>999.999	>999.999	>999.999	<0.0001
Mid diameter	>999.999	369.749	>999.999	<0.0001
Base diameter	>999.999	>999.999	>999.999	<0.0001
Milk leakage	2.813	1.383	5.724	<0.005
Udder shape	2.574	1.904	3.480	<0.0001
Pendulous udder	2.549	1.489	4.363	<0.001
Feeding systems	2.040	1.245	3.340	<0.005
Udder depth	2.012	1.637	2.473	<0.0001
Teat shape	1.940	1.573	2.393	<0.0001
Calf suckling	1.440	1.275	1.706	<0.001
Milk yield	1.230	1.093	1.385	<0.001
Teat lesions	1.157	1.035	1.295	<0.01
Live body weight	1.013	1.009	1.018	<0.0001
Multivariate logistic regression including farm and individual variable in the model				
Teat length	0.009	0.003	0.027	<0.0001
Frequency of culling	<0.001	<0.001	<0.001	<0.0001
Floor distance	0.494	0.424	0.576	<0.0001
Number of attendants	0.941	0.889	0.996	<0.05
Teat involved	>999.999	117.337	>999.999	<0.005
Apex diameter	>999.999	>999.999	>999.999	<0.0001
Mid diameter	>999.999	426.553	>999.999	<0.0001
Milk leakage	2.822	1.386	5.745	<0.005
Feeding systems	2.275	1.348	3.841	<0.005
Udder shape	2.580	1.907	3.489	<0.0001
Pendulous udder	2.548	1.489	4.362	<0.001
Udder depth	2.041	1.655	2.518	<0.0001
Teat shape	1.945	1.576	2.400	<0.0001
Calf suckling	1.414	1.252	1.679	<0.0005
Milk yield	1.235	1.095	1.391	<0.001
Teat lesions	1.157	1.035	1.295	<0.01
Live body weight	1.018	1.013	1.023	<0.0001
Multivariate logistic regression including breed, age and individual variable in the model				
Teat length	0.005	0.002	0.017	<0.0001
Floor distance	0.439	0.367	0.524	<0.0001
Number of attendants	0.916	0.840	0.998	<0.05
Teat involved	>999.999	116.913	>999.999	<0.005
Apex diameter	>999.999	>999.999	>999.999	<0.001
Pendulous udder	2.589	1.510	4.440	<0.0005
Udder shape	2.575	1.902	3.486	<0.0001
Milk leakage	2.760	1.352	5.632	<0.01
Feeding systems	2.241	1.326	3.788	<0.005
Udder depth	2.058	1.660	2.550	<0.0001
Lactation stage	1.941	1.899	1.985	<0.0001
Teat shape	1.937	1.568	2.393	<0.0001
Milk yield	1.236	1.086	1.406	<0.005
Calf suckling	1.402	1.244	1.661	<0.0005
Teat lesions	1.161	1.037	1.299	<0.01
Live weight	1.018	1.013	1.023	<0.0001

found an association of teat length and teat diameter with occurrence of mastitis (Slettbakk *et al.*, 1995; Chrystal *et al.*, 1999). These results suggest that the chances of mastitis are higher if the teat length is shorter and if the teat diameter is greater. The results of present study suggested that there is no breed association as the mastitis can occur in both Sahiwal breed, Cross-bred and non-descript cattle at almost same rate and there is also no difference in occurrence of mastitis in animals with relation to housing space provided. This was in line with previous report where also no effect of space has been found in relation to mastitis (Fregonesi and Leaver, 2001).

Present results also suggested strong association of lactation stage, udder shape, teat shape, teat lesions,

feeding system, milk leakage from teat and use of oxytocin with occurrence of mastitis. The mastitis in cattle was higher in animals in their third lactation, having round/bowl shaped udder, cylindrical/flat/round teats, who were stall fed along with grazing and where oxytocin was frequently used. As the orifice is the first line of defense against invading pathogens, lesions or damage to this part may reduce its effectiveness in preventing mastitis (Gleeson *et al.*, 2004). In present study among different teat and udder lesions udder edema was an important risk factor for mastitis which may impairs milk removal (Waage *et al.*, 2001; Bhutto *et al.*, 2010). The higher prevalence of mastitis was in cattle having round and pendulous udder. It may be due to the reason that long and pendulous udder gets injuries and helps the pathogens to grow. The higher prevalence of mastitis in advanced lactation period and parity may be due to the removal of protective keratin “plug” in teats (Dingwell *et al.*, 2004), and reduction in teat end to floor space may causes contamination of teat and udder which support bacterial invasions into mammary gland (Slettbakk *et al.*, 1990). The use of oxytocin may have been linked to the opening of teat orifice which remains open for longer time and the teat muscles remain in relaxed position thus enabling the easy entry of pathogens to the udder eventually leading to mastitis.

Conclusion: Some risk factors are negatively, while others are positively associated with mastitis. The association of age was not found, while udder depth and use of oxytocin was found associated with mastitis along with other known risk factors.

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