Pakistan J. Zool., vol. 48(2), pp. 519-525, 2016.

Prevalence and Association of Possible Risk Factors with Sub-Clinical Mastitis in Cholistani Cattle

Abdul Qayyum[1, 2], Jawaria Ali Khan[1], Riaz Hussain[2], Muhammad Avais[1], Nisar Ahmed[3], Ahrar Khan[4]* and Muhammad Sarwar Khan[1]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-63100, Bahawalpur, Pakistan.
[3]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Pathology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

The current study was planned to investigate the potential risk factors and their association with mastitis in Cholistani cattle. For this purpose milk samples were collected and examined for mastitis using California Mastitis Test. The results showed an overall 21.96% prevalence of subclinical mastitis. The analysis of variance did not show significant association of tail size, live body weight, mid and base teat diameter with mastitis. The analysis of variance showed significantly higher involvement of right rear quarters. Results showed that teat length, teat end to floor distance and milk yield (P<0.001) were significantly lower in mastitic animals while teat apex diameter, parity and udder depth was significantly higher (P<0.001). The frequency analysis revealed significant association of mastitis between different groups such as age, udder depth, lactation stage, parity, teat shape, udder shape, teat lesions and live body weight (P<0.001). Bivariate logistic regression analysis indicated significant association of age, parity, lactation stage, teat shape, udder depth, teat and udder lesions, teat end to floor distance, milking technique, milk leakage and type of floor with mastitis.

Article information
Received 24 June 2015
Revised 14 September 2015
Accepted 1 October 2015
Available online 1 March 2016

Authors' Contributions
AQ, JAK and RH conceived the idea and tailored the projected. AQ, JAK, RH, NA and MSK executed the project and collected the data. RH, AK and MA analyzed the data statistically. AQ, JAK and MSK wrote the article.

Key words
Cattle, sub clinical mastitis, prevalence, Risk factors.

INTRODUCTION

In Pakistan dairy animals play an important role in the economy of the people in terms of milk and meat production and are usually kept in small groups (Mufti *et al*., 2014). Pakistan is ranked at the 3rd largest milk producing country in the world (Abbas *et al*., 2014), however, several disease problems occur in dairy animals (Brown *et al.*, 2014; Mahmood *et al.*, 2014) which impede its milk production. Among these, mastitis is one of the most important diseases hampering the growth of the dairy sector (Karahan *et al.*, 2011; Khan *et al.*, 2013). It causes huge economic loss in dairy animals because of lower milk production, high treatment costs, milk withdrawal following treatment, labor, premature culling and death (Yang *et al.*, 2011). Long term production losses are incurred at the initial phase of lactation due to mastitis (Dieser *et al.*, 2014).

Mastitis, a continuous threat to dairy industry, is a multifaceted disease that occurs as acute, chronic, gangrenous and sub-clinical forms. It is a devastating and complex disease of lactating animals (Elbably *et al.*, 2013; Tripura *et al.*, 2014) which is characterized by inflammation of the mammary parenchyma (Zenebe *et al.*, 2014). Infectious agents, animal hygiene, management and environment factors play important role in establishing the infection in the udder (Kulkarni and Kaliwal, 2013; Hussain *et al.*, 2014a).

The incidence of mastitis is greatly influenced by environment and management related factors (Steeneveld *et al.*, 2008; Ali *et al.*, 2014). Pakistan being an agricultural country has tropical and subtropical environment (Khan *et al*., 2011; Hussain *et al.*, 2014b). Both these environments favor the growth of organisms responsible for mastitis (Hussain *et al.*, 2013). Various microbial agents first get entry into teat canal, multiplies in mammary parenchyma, inflammation starts and the resultant is the mastitis (Memon *et al*., 2013).

Prevalence of mastitis is considerably increased and is a prime challenge for veterinarians and researchers (Awale *et al.*, 2012). Numerous risk factors with bovine mastitis are connected as microflora of the udder, udder shape, udder condition, teat injuries, teat length, increasing teat canal diameter, udder depth, teat morphology (Tiwari *et al.*, 2013; Ali *et al.*, 2014). Scanty information is available about the prevalence of subclinical mastitis and association of potential risk factor in Cholistani cattle being extensively reared for milk production in desert conditions. The present study was planned to investigate the prevalence and possible risk factors of mastitis in Cholistani cattle.

* Corresponding authors: ahrar1122@uaf.edu.pk
0030-9923/2016/0002-0519 $ 8.00/0

MATERIALS AND METHODS

Study area, animals and management

The present study was carried out from November 2013 to December 2014, at district Bahawalpur, which is located at longitude 71.68°E, latitude 29.39°N and altitude 152 m. The environmental temperature of that area varies from 40 to 50 °C with 63.1% relative humidity (Khalid and Gilani, 2010). A total of 1457 lactating Cholistani cattle, kept under different housing and management practices in the desert and rural areas, were selected in and around Bahawalpur. In this area the Cholistani cattle are mainly reared for milk and socioeconomic purpose in desert conditions by pastoralist (Ali *et al.,* 2009; Farooq *et al.,* 2010).

Detection of sub-clinical mastitis

All the lactating cattle after one month of calving were screened for subclinical mastitis. Milk samples were collected from all lactating cows of different age, parity and stage of lactation. About 10 ml milk sample was collected aseptically after discarding the first few streams of milk from each quarter (Anonymous, 1990) and tested with California mastitis test (Schalm *et al.,* 1971). All the milk samples with precipitates were considered as positive and those free from any precipitation were considered as negative. The California mastitis test was considered as a response variable.

Sampling strategy and risk factors

A total of 1457 cows were sampled through cluster sampling to study the various risk factors associated with sub-clinical mastitis. In this study 70 villages along with 25 tobas of Cholistan were selected randomly by keeping one village/Toba as a single cluster with a minimum of 10 lactating Cholistani cows in each cluster. All the lactating Cholistani cows present at Livestock Experimental Station, Jugait Peer were also screened for sub-clinical mastitis. Data regarding various risk factors for each animal related to animals and farm/surroundings, including age, parity, stage of lactation, teat length, teat diameter, teat end to floor distance, milk leakage, live body weight, calf suckling, use of oxytocin, milk yield and milking techniques were recorded. The data about teat/udder lesions, pendulous/non-pendulous udder, udder depth, teat and udder shape, frequency of culling, tail length were also recorded. The teat diameter and teat length was measured with vernier calipers, while all other measurements were made with the help of measuring tape (Hussain *et al.,* 2013).

Statistical analyses

The data collected was analysed by using SAS 9.1 statistical software (SAS, 2004). Data of parity, age, live body weight, tail length, teat to floor distance, udder depth, milk yield, teat length and teat diameter (apex, mid and base) were analyzed by analysis of variance technique and means were compared by t-test. Data on these and some of the other variables were analyzed by using Mantel-Hanszel chi-square and logistic regression (bivariate) procedures. The bivariate logistic analysis was carried out by taking a single variable in the model with mastitis as an outcome variable. In the bivariate frequency procedure, where possible the appropriate odds ratio and 95% confidence limits were also worked out (Hussain *et al.,* 2013).

Table I.- Prevalence of subclinical mastitis in Cholistani cows kept at different private and public livestock herds.

Herd type	Total examined	Positive	% prevalence	95% CI
Private herds				
Villages	1092	242	22.2	19.77-24.7
Tobas	315	68	21.6	17.31-26.39
Total	1407	310	22.0	19.92-24.26
Public Livestock farm				
Jugait Peer Farm	50	10	20.0	10.63-32.76
Overall	1457	320	21.96	19.89-24.14

RESULTS

The results showed an overall 21.96% prevalence of subclinical mastitis in Cholistani cows. The results revealed non-significant difference in prevalence of mastitis between public and private dairy herds (Table I). The results about quarter based prevalence are presented in Table II. Results showed that from a total of 1457 cattle an overall 7.88% of quarters were infected with subclinical mastitis. The prevalence of mastitis was significantly higher in right rear quarters ($P<0.001$).The results of the present study showed a total of 2.93% quarters were blocked with involvement of significantly higher prevalence of right rear quarters (5.91%) due to chronic infection (Table III). Udder depth, ($P<0.001$) teat end to floor distance and milk yield were significantly lower in mastitic animals while parity and udder depth was significantly higher as compared to healthy animals. Teat length was significantly lower and the teat apex diameter was higher in all the quarters of mastitic than healthy cattle. The teat mid-diameter and teat base diameter in all quarters was non-significantly different in mastitic and healthy quarters (Table IV).

Table II.- Quarter based prevalence of sub-clinical mastitis in Cholistani cows.

Quarter	No. Positive (CMT 1+ to CMT 3+)	Total examined	% preva-lence	95% CI
LR	114	1433	7.96	6.64- 9.44
LF	93	1438	6.46	5.28- 7.83
RR	145	1371	10.57	9.03-12.29
RF	94	1415	6.64	5.43- 8.03
Overall	446	5657	7.88	7.20- 8.61

LR, left rear; LF, left front; RR, right rear and RF, right front.

Table III.- Prevalence of blocked quarters due to previous untreated clinical mastitis in Cholistani cows.

Quarters involved	No. of Blocked quarters	Total examined	% prevalence	95% CI
LR	24	1457	1.65	1.1-2.4
LF	19	1457	1.31	0.9-1.9
RR	86	1457	5.91	4.7-7.2
RF	42	1457	2.88	2.1-3.8
Total	171	5828	2.93	2.5-3.39

For abbreviations, see table II.

The frequency analysis revealed significant association of age, udder depth, lactation stage ($P<0.001$), parity, teat lesions, live body weight, teat and udder shape between healthy and mastitic animals. The prevalence of mastitis was higher in older animals, cattle at their third stage of lactation, in 3rd parity, in cattle having increased udder depth ($P<0.001$) and having various teat and udder lesions (Table 5). Results indicate increased prevalence of mastitis in cattle with cup shape udder, pointed teat, dirty hind legs, in chained animals and the animals which were kept on brick or cemented floor. The bivariate logistic analysis revealed a protective effect of decreased udder depth, during mid stage of lactation, with cylindrical teat shape and round shape udder against mastitis. While age, parity, milk leakage from the teats, lactation stage, teat shape, udder depth teat lesions, teat end to floor distance and milking technique were significantly ($P<0.001$) associated with mastitis (Table VI).

DISCUSSION

The livestock sector in Pakistan is crucial and play important role in the economy of the people with an estimated heads of 33.0 million cattle (Bachaya *et al.*, 2011). In Pakistan, about 95% milk is produced by cattle and buffaloes (Hussain *et al.*, 2012a; Ahmad *et al*., 2014).

Table IV.- Comparison of various physical parameters (Mean±SD) by analysis of variance between healthy and mastitic Cholistani cattle.

Parameters	Healthy	Mastitic	P-Value
Age (Years)	6.75±2.79	8.97±4.57	<0.051
Parity	3.02±2.26	3.81±2.83	<0.0001
Tail size (cm)	99.01±5.08	98.99±5.39	>0.791
Live body weight (Kg)	338.25±41.87	342.04±40.78	>0.15
Milk yield (L)	9.49±1.87	7.77±1.54	<0.0001
Teat to floor distance (cm)	44.17±2.40	33.55±3.12	<0.0001
Udder depth (cm)	11.80±1.50	15.38±1.29	<0.0001
Teat length (cm)			
Right rear	5.44±1.01	4.56±0.45	<0.0001
Left rear	5.53±1.03	4.56±0.47	<0.0001
Right front	6.21±1.14	4.89±0.65	<0.0001
Left front	6.24±1.2	4.93±0.63	<0.0001
Teat apex diameter (cm)			
Right Rear	0.68±0.11	0.82±0.12	<0.0001
Left Rear	0.67±0.12	0.82±0.12	<0.0001
Right Front	0.68±0.11	0.83±0.13	<0.0001
Left Front	0.68±0.11	0.83±0.12	<0.0001
Mid teat diameter (cm)			
Right rear	2.30±0.31	2.30±0.31	>0.927
Left rear	2.32±0.29	2.31±0.29	>0.874
Right front	2.31±0.30	2.30±0.29	>0.997
Left front	2.35±0.89	2.32±0.32	>0.665
Teat base diameter (cm)			
Right rear	3.12±0.57	3.11±0.59	>0.868
Left rear	3.18±0.51	3.15±0.54	>0.382
Right front	3.14±0.53	3.16±0.54	>0.578
Left front	3.20±0.49	3.19±0.52	>0.678

This country has been gifted with the best breeds of all kinds of animals and Cholistani breed of cattle is one of the famous dual purpose cattle breed with its habitat in a widespread desert of Cholistan situated in the Bahawalpur region of the southwest Punjab province (Abdullah *et al.*, 2013; Rafay *et al*., 2013). The only feeding source for the livestock in Cholistan is the patchy vegetative growth, holophytic plants & trees/bushes. In spite of an uncertain, unpredictable rainfall, low humidity and extremes in temperatures, Cholistan has long been famous for raising different breeds of livestock and contributing a significant share to national milk and meat production (Ali *et al.*, 2009).

Numerous diseases are responsible for reduction in milk production, among these mastitis is the most important and various contributory factors have been reported, but such reports are scanty in Cholistani Cattle. Therefore, it was necessary and crucial to know the prevalence of mastitis and different extrinsic and intrinsic

Table V.- Bivariate frequency analysis of different variables in healthy and mastitic Cholistani cows.

Parameters	Positive		Negative	95% CL	MH Chi-sq P value
	n	%			
Stage of lactation (Months)					
>0.9-3	80	18.7	348	15.2- 22.6	0.001
>3-6	80	13.9	495	11.3- 16.9	
>6	160	35.2	294	30.9- 39.7	
Udder depth (cm)					
10-12	25	11.6	190	7.84- 16.5	0.001
13-15	225	23.0	753	20.45 - 25.7	
16-18	70	27.2	187	22.06 - 32.9	
Parity					
1-3	186	19.5	770	17.04 - 22.1	0.001
4-6	70	20.1	279	16.10- 24.5	
7-9	55	45.8	65	37.07- 54.8	
>10	9	28.1	23	14.67 - 45.4	
Age (years)					
3-6	151	19.5	623	16.83- 22.41	0.001
6.1-9	45	23.0	151	17.48- 29.24	
9.1-12	41	17.7	190	13.22- 23.08	
>12	83	32.4	173	26.90- 38.34	
Teat shape					
Cylindrical	97	15.9	512	13.18 - 19.00	0.032
Pointed	131	33.9	255	29.34 - 38.77	
Flat	39	17.3	187	12.74 - 22.60	
Round	53	22.5	183	17.47 - 28.11	
Body weight (Kg)					
>220	5	23.8	16	9.29 - 45.15	0.05
>270	122	20.0	488	16.97 - 23.32	
>320	110	22.7	374	19.16 - 26.62	
>370	83	24.3	259	19.95 - 29.03	
Teat lesions					
None	177	16.9	869	14.74- 19.29	0.001
Laceration	57	37.3	96	29.86- 45.12	
Abrasion	43	47.8	47	37.61- 58.09	
Inflammation	3	10.7	25	2.80- 26.45	
Cord formation	5	38.5	8	15.68- 65.91	
Hemorrhages	23	31.9	49	21.97- 43.35	
Necrosis	10	25.0	30	13.45 - 40.05	
Edema	2	13.3	13	2.30 - 37.52	
Udder shape					
Cup	194	23.7	624	20.90 - 26.72	0.02
Bowl	73	20.3	287	16.36 - 24.67	
Round	53	19.0	226	14.71- 23.92	

risk factors which favor the entry of different pathogens in the udder. In the present study, overall 21.9% prevalence of mastitis at cow level and 31.6% at quarter level was recorded. In the past, increased prevalence of subclinical mastitis in cattle and buffaloes in Pakistan has been reported (Mustafa *et al.*, 2011; Akhtar *et al.*, 2012; Hameed *et al.*, 2012). However, lower prevalence of mastitis in cattle and buffaloes in Pakistan (Hussain *et al.*,

2012a) and different other regions have been reported (Hunderra *et al.,* 2005; Bhatt *et al.,* 2011). Various reports are available about the increased prevalence of sub clinical mastitis in cattle (Khanal and Pandit, 2013; Elbably *et al.,* 2013; Zeryehun *et al.,* 2013; Rahman *et al.,* 2014). The difference in prevalence of subclinical mastitis may be due to the different husbandry practices, diagnostic techniques, environmental conditions and immune status of animals. The prevalence of mastitis was significantly associated with age. However, non-significant association of mastitis with age has been recorded (Hussain *et al.,* 2013), while significant association of age with mastitis was reported (Moges *et al.,* 2011; Zeryehun *et al.,* 2013). The increased prevalence of mastitis in older animals in this study can be related to increased susceptibility of pathogenic organisms in udder relaxed sphincter muscles of teats. The prevalence of mastitis was significantly higher in right rear quarters. Increased prevalence of mastitis in hind quarters might be due to increased milk production performance followed with relaxed teat sphincters and contaminated hind legs. These results are supported by various other workers who also reported increased prevalence of mastitis in rear quarters (Zeryehun *et al.,* 2013; Tripura *et al.,* 2014; Zenebe *et al.,* 2014). Results revealed that the prevalence of mastitis was significantly higher in small quarters and teats having an increased apex diameter. These findings are in agreement with the previous results (Bhutto *et al.,* 2010; Hussain *et al.,* 2012b). The prevalence of mastitis was significantly increased in animals having higher udder depths, parity and lower teat end to floor distance. A significant association of increased udder depth, parity and lower teat end to floor distance with mastitis has been reported (Kathiriya *et al.,* 2014). The lactation stage was significantly associated with mastitis in this study as reported earlier (Kayesh *et al.,* 2014; Adane *et al.,* 2012). However, different studies showed higher prevalence of mastitis in early stage of lactation (Girma *et al.,* 2012). In the present study the analysis of variance did not show a significant association of tail length and body weight with mastitis. However, the frequency analysis indicated a significant association of increased body weight with mastitis. Similar reports are also available (Bedacha and Menghistu, 2011; Hussain *et al.,* 2013). Results also showed a significant association of teat shape, udder shape (Klaas *et al.,* 2004; Bhutto *et al.,* 2010) and teat lesions between healthy and mastitic animals. The logistic analysis also showed significant association of these parameters with mastitis. The Association of different udder and teat traits have also been reported (Bedacha and Menghistu, 2011; Deng *et al.,* 2012). The bivariate logistic analysis also revealed significant association of milk leakage, teat lesions, milking technique, housing of animals and floor type.

Table VI.- Results of bivariate logistic regression analysis.

Variables	Odds ratio	Lower-Upper (95% CL)	P value
Lactation stage	3.07	2.06-4.57	0.001
Milking technique	8.3	4.18-5.89	0.001
Udder depth	3.22	1.79-5.8	0.001
Teat lesions	0.32	0.17-0.57	0.001
Teat end to floor distance	5.59	2.54-12.28	0.001
Parity	2.50	1.0-6.09	0.043
Teat shape	2.01	1.33-3.02	0.001
Age	3.52	1.34-9.23	0.011
Milk leakage	2.20	1.39-3.47	0.001
Floor types	2.99	2.10-4.25	0.001

ACKNOWLEDGEMENTS

The grant provided by Higher Education Commission, Islamabad, Pakistan vide Project No. PM-IPFP/HRD/HEC/2012/3524 entitled "Molecular epidemiology of Sub-clinical mastitis in Cholistani cattle at a public farm and different private cattle herds in and around District Bahawalpur" is highly acknowledged.

REFERENCES

Abbas, N., Zahoor, T. and Rahman, Z.U., 2014. Physicochemical profile of milk from oxytocin injected buffaloes. *Pakistan Vet. J.*, **34**: 351-355.

Abdullah, M., Khan, R.A., Yaqoob, S. and Ahmad, M., 2014. Community structure of browse vegetation in Cholistan rangelands of Pakistan. *Pakistan J. agric. Sci.*, **50**: 237-247.

Adane, B., Guyo, K., Tekle, Y., Taddele, H., Bogale, A. and Biffa, D., 2012. Study on prevalence and risk factors of bovine mastitis in Borana pastoral and agro-pastoral settings of Yabello district, Borana zone, Southern Ethiopia. *Eurasian J. Agric. environ. Sci.*, **12**: 1274-1281.

Ahmad, Z., Babar, S., Abbas, F., Awan, M.A., Shafee, M., Tariq, M.M., Alam, M., Rashid, N., Amin, S., Taj, K. and Ali, M., 2014. Prevalence of *Mycoplasma bovis* in respiratory tract of cattle slaughtered in Balochistan, Pakistan. *Pakistan Vet. J.*, **34**: 46-49.

Akhtar, A., Umer, K.B., Tanweer, A.J. and Habibullah, 2012. Prevalence of mastitis and identification of causative pathogens in local and crossbred cows in Dera Ismail Khan. *Pakistan J. Sci.*, **64**: 265-268.

Ali, I., Chauhdry, M.S. and Farooq, U., 2009. Camel rearing in Cholistan desert of Pakistan. *Pakistan Vet. J.*, **29**: 85-92.

Ali, T., Rahman, A., Qureshi, M.S., Hussain, M.T., Khan, M.S., Din, S.U., Iqbal, M. and Han, B., 2014. Effect of management practices and animal age on incidence of

mastitis in Nili Ravi buffaloes. *Trop. Anim. Hlth. Prod.*, **46**: 1279–1285.

Anonymous, 1990. *Microbiological procedures for the diagnosis of bovine udder infection.* National Mastitis Council Inc., Arlington, Virginia, USA

Awale, M.M., Dudhatra, G.B., Avinash, K., Chauhan, B.N., Kamani, D.R., Modi, C.M., Patel, H.B. and Kennedy, R., 2012. Bovine mastitis: a threat to economy. *Open Access Sci. Rep.*, **1**: 295.

Bachaya, H.A., Raza, M.A., Murtaz, S. and Akbar, I.U.R., 2011. Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). *J. Anim. Pl. Sci.*, **21**: 16-19.

Bedacha, B.D. and Menghistu, H.T., 2011. Study on prevalence of mastitis and its associated risk factors in lactating dairy cows in Batu and its Environs, Ethiopia. *Global Vet.*, **7**: 632-637.

Bhatt, V.D., Patel, M.S., Joshi, C.G. and Kunjadia, A., 2011. Identification and antibiogram of microbes associated with bovine mastitis. *Anim. Biotech.*, **22**: 163-169.

Bhutto, A.L., Murray, R.D. and Woldehiwet, Z., 2010. Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. *Vet. J.*, **183**: 63–67.

Brown, C., Garin-Bastuji, B., Ziay, G., Hayajneh, W., Al-Fuqaha, S., Hemida, H., Otify, Y., Hailat, I., Alkhlouf, S. and Hailat, H., 2014. Improving the diagnostic capacities of brucellosis, enhancing the vaccination and control strategies in the Middle East and North Africa. *Pakistan Vet. J.*, **34**: 263-264.

Deng, M.P., Badri, T.M., Atta, M. and Hamad, M.E., 2012. Relationship between udder dimensions and milk yield of Kenana × Friesian crossbred cows. *Res. Opin. Anim. Vet. Sci.*, **2**: 49-54.

Dieser, S.A., Vissio, C., Lasagno, M.C., Bogni, C.I., Larriestra, A.J. and Odierno, L.M., 2014. Prevalence of pathogens causing subclinical mastitis in Argentinean dairy herds. *Pakistan Vet. J.*, **34**: 124-126.

Elbably, M. A., Emeash, H.H. and Asmaa, N.M., 2013. Risk factors associated with mastitis occurrence in dairy herds in Beni Suef Governorate. *World's Vet. J.*, **3**: 05-10.

Farooq, U., Samad, H.A., Sher, F., Asim, M. and Khan, M.A., 2010. Cholistan and Cholistani breed of cattle. *Pakistan Vet. J.*, **30**: 126-130.

Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. and Jibat, T., 2012. Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *J. Vet. Med. Anim. Hlth.*, **4**: 116-123.

Hameed, S., Arshad, M., Ashraf, M., Avais, M. and Shahid, M.A., 2012. Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of tehsil Burewala, Pakistan. *J. Anim. Pl. Sci.*, **22**: 371-376.

Hunderra, B., Etana, D., Ademe, Z. and Sintayehu, A., 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. *Int. J. appl. Res. Vet. Med.*, **3**: 432.

Hussain, R., Khan, A., Javed, M.T. and Rizvi, F., 2012b. Possible risk factors associated with mastitis in indigenous cattle in Punjab, Pakistan. *Pakistan Vet. J.*, **32**: 605-608.

Hussain, R., Javed, M.T. and Khan, A., 2012a. Changes in some biochemical parameters and somatic cell counts in the milk of buffalo and cattle suffering from mastitis. *Pakistan Vet. J.*, **32**: 418-421.

Hussain, R., Javed, M.T., Khan, A. and Muhammad, G., 2013. Risks factors associated with sub-clinical mastitis in water buffaloes in Pakistan. *Trop. Anim. Hlth. Prod.*, **45**: 1723-1729.

Hussain, R., Mahmood, F., Khan, A., Khan, M. Z. and Siddique, A.B., 2014a. Pathological and molecular based study of pneumonic pasteurellosis in cattle and buffalo (*Bubalus bubalis*). *Pakistan J. agric. Sci.*, **51**: 235-240.

Hussain, R., Javed, M.T., Mahmood, F., Hussain, T., Chaudhry, H.R., Aslam, M.S., Ghori, M.T., Qayyum, A., Babar, W., Hameed, S. and Rehman, A.U., 2014b. Clinico-pathologic findings of enterotoxemia in Chinkara deer (*Gazella bennettii*) under desert conditions in Pakistan. *Pakistan Vet. J.*, **34:** 400-402.

Karahan, M., Acik, M.N. and Cetinkaya, B., 2011. Investigation of virulence genes by PCR in *Stapylococcus aureus* isolates originated from subclinical bovine mastitis in Turkey. *Pakistan Vet. J.*, **31**: 249-253.

Kathiriya, J.B., Kabaria, B.B., Saradava, D.A. and Sanepara, D.P., 2014. Prevalence of subclinical mastitis in dairy cows in Rajkot district of Gujarat. *Int. J. Sci. Nature*, **5**: 433-436.

Kayesh, M.E.H., Talukder, M. and Anower, A.K.M.M., 2014. Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. *Int. J. biol. Res.*, **2**: 35-38.

Khalid, S. and Gilani, A.H., 2010. Distinctive cultural and geographical legacy of Bahawalpur. Pakistan. *J. Pak. Stud.*, **2**:1-17.

Khan, A., Saleemi, M.K., Khan, M.Z., Gul, S.T., Irfan, M. and Qamar, M.S., 2011. Hemorrhagic septicemia in buffalo (*Bubalus bubalis*) calves under sub-tropical conditions in Pakistan. *Pakistan J. Zool.*, **43**: 295–302.

Khan, A., Hussain, R., Javed, M.T. and Mahmood, F., 2013. Molecular analysis of virulent genes (*coa* and *spa*) of *staphylococcus aureus* involved in natural cases of bovine mastitis. *Pakistan J. agric. Sci.*, **50**: 739-743.

Khanal, T. and Pandit, A., 2013. Assessment of sub-clinical mastitis and its associated risk factors in dairy livestock of Lamjung Nepal. *Int. J. Infect. Microbiol.*, **2**: 49-54.

Klaas, I.C., Enevoldsen, C., Vaarst, M. and Houe, H., 2004. Systematic clinical examinations for identification of latent udder health types in Danish dairy herds. *J. Dairy Sci.*, **87**: 1217–1228.

Kulkarni, A.G. and Kaliwal, B.B., 2013. Bovine mastitis: A review. *Int. J. Rec. Sci. Res.*, **5**: 543- 548.

Mahmood, F., Khan, A., Hussain, R. and Khan, I.A., 2014.

Molecular based epidemiology of bovine pulmonary tuberculosis – a mortal foe. *Pakistan Vet. J.*, **34**: 185-188.

Memon, J., Kashif, J., Yaqoob, M., Liping, W., Yang, Y. and Hongjie, F., 2013. Molecular characterization and antimicrobial sensitivity of pathogens from sub-clinical and clinical mastitis in Eastern China. *Pakistan Vet. J.*, **33**: 170-174.

Moges, N., Asfaw, Y. and Belihu, K., 2011. Cross sectional study on the prevalence of sub clinical mastitis and associated risk factors in and around Gondar Northern Ethiopia. *Int. J. Anim. Vet. Adv.*, **3**: 455-459.

Mufti, S., Afshan, K., Khan, I.A., Zafar, Y., Rizvi, S.S.R., Nazir, F. and Qayyum, M., 2014. Genetic characterization of *Fasciola* samples from bovine hosts in Pakistan by sequences of ribosomal internal transcribed spacer regions. *Pakistan Vet. J.*, **34**: 361-366.

Mustafa, Y.S., Awan, F.N., Zaman, T., Chaudhry, S.R. and Zoyfro, V., 2011. Prevalence and antibacterial susceptibility in mastitis in buffalo and cow in and around the district Lahore-Pakistan. *Pakistan J. Pharm.*, **24**: 29-33.

Rafay, M., Khan, R.A., Yaqoob, S. and Ahmad, M., 2014. Floristic composition of grass species in the degrading rangelands of Cholistan desert. *Pakistan J. agric. Sci.*, **50**: 599-603.

Rahman, M.M., Munsi, M.N., Ekram, M.F., Kabir, M.H., Rahman, M.T. and Saha, S., 2014. Prevalence of subclinical mastitis in cows at Anwara, a Coastal Upazila of Chittagong district in Bangladesh. *J. Vet. Adv.*, **4**: 594-598.

SAS, 2004. *SAS Statistical Software Version 9.1*. SAS Institute Inc, Cary, NC, USA

Schalm, O.W., Carol, E.J. and Jain, N.C., 1971. *Bovine mastitis*. Lea and Febiger, Philadelphia, USA, pp. 128-157.

Steeneveld, W., Hogeveen, H., Barkema, H.W., Broek, V.D. and Huirne, B.M., 2008. The influence of cow factors on the incidence of clinical mastitis in dairy cows. *J. Dairy Sci.*, **91**: 1391-1402.

Tiwari, J.G., Babra, C., Tiwari, H.K., Williams, V., Wet, S.D., Gibson, J., Paxman, A., Morgan, E., Costantino, P, Sunagar, R., Isloor, S. and Mukkur, T., 2013. Trends in therapeutic and prevention strategies for management of bovine mastitis: An overview. *J. Vaccines Vaccin*, **4**: 1000176.

Tripura, T.K., Sarker, S.C., Roy, S.K., Parvin, M.S., Sarker, R.R., Rahman, A.K.M.A. and Islam, M.T., 2014. Prevalence of subclinical mastitis in lactating cows and efficacy of intramammary infusion therapy. *Bangladesh J. Vet. Med.*, **12**: 55-61.

Yang, F.L., Li, X.S., He, B.X., Yang, X.L., Li, G.H., Liu, P., Huang, Q.H., Pan, X.M. and LI, J., 2011. Malondialdehyde level and some enzymatic activities in subclinical mastitis milk. *Afr. J. Biotech.*, **10**: 5534-5538.

Zenebe, N., Habtamu, T. and Endale, B., 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *Afr. J. Microbiol. Res.*, **8**: 327-331.

Zeryehun, T., Aya, T. and Bayecha, R., 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *J. Anim. Pl. Sci.*, **23**: 50-55.