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Epidemiology of Bovine Sub-Clinical Mastitis in Pothohar Region, Punjab, Pakistan in 2018

Asghar Khan[1,*], Aneela Zameer Durrani[1], Arfan Yousaf[2], Jawaria Ali Khan[1], Mamoona Chaudhry[1], Mumtaz Ali Khan[3], Habibunnabi[4] and Amjad Khan[5]

[1]*University of Veterinary and Animal Sciences, Lahore*
[2]*Pir Mehr Ali Shah ARID Agricultural University, Rawalpindi*
[3]*Civil Veterinary Hospital, Mardan*
[4]*Veterinary Research Institute, Swat*
[5]*Pakistan Kidney and Liver Institute and Research Center, Lahore*

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AK executed the experimental work and wrote the article. AZD supervised the work and helped in write up and diagnostics. JAK, MC and AK analyzed the data. AY and MAK contributed in the field work and study design development. MAK actively contributed in the field work. H contributed in the diagnostic process.

Key words
Sub-clinical mastitis, Prevalence, Risk factors, California mastitis test.

ABSTRACT

Sub-clinical mastitis considered as a leading hindrance in the development of dairy industry in Pakistan. Pakistan is the top listed country with the highest prevalence of Sub-clinical mastitis. Therefore, this study was conducted to estimate the prevalence and assess the potential risk factors of sub-clinical mastitis in lactating buffaloes in Pothohar region of Pakistan. A questionnaire based cross-sectional based survey was conducted in 30 commercial and subsistent farms in Pothohar region of Rawalpindi district, Punjab. Where randomly 196 lactating buffaloes were sampled. Data on several health, management and bio-security variables was collected in face to face interviews from farmers, managers and owners. Milk samples collected were subjected to California Mastitis Test.Data entry and validation was performed through Epi-Data. Data analysis was performed through SPSS. Chi-square and regression analysis were conducted. An overall prevalence of 67.3% was found. On multivariable logistic regression several health (lactation stage, number of lactations, body mass index, udder shape and milk yield), management (udder preparation and teat dipping) and bio-security (source of animal, feed sharing, manure removal and de-worming) factors were identified as potential risk factors (OR>1; P-Value<0.05). An increasing trend of prevalence of sub-clinical mastitis was observed considering previous studies from Pakistan. It can be concluded that the health, management and bio-security measures are under satisfactory level. To control the epidemic trend of sub-clinical mastitis in Pakistan addressing these factors needs to be prioritized.

INTRODUCTION

Mastitis is an important disease of cattle and buffaloes globally forcing large economic losses (Kumar *et al*., 2011) by causing parenchymal mammary inflammation (Zenebe *et al*., 2014). Mastitis occurs in several forms such as per-acute, acute, chronic, sub-clinical and gangrenous being devastating of complex nature in all forms of dairy animals (Elbably *et al*., 2013; Tripura *et al*., 2014). Subclinical mastitis refers to mammary gland inflammation with no signs of gross lesions in udder (Harmon, 1994). Therefore, early detection of subclinical mastitis is not possible without continuous monitoring; making it more severe than the clinical mastitis (Begum *et al*., 2015). Cattle infected with subclinical mastitis remains constant reservoir of the causing pathogens leading to udder infection and its spread in the premises (Tiwari *et al*., 2013).

Pakistan is an agricultural country; with large population of cattle (46.1 million) and buffaloes (38.8 million) (GoP, 2018). Livestock as agriculture subsector contributes 56% of value addition in it and approximately 11% to national gross domestic product (Rehman *et al*., 2017). Dairy animals in Pakistan faces several diseases all the times; amongst which mastitis is the high burden and deterring factor in the development of dairy sector (Khan *et al*., 2015). It causes decrease in milk production, condemnation of milk, replacement of animals and their culling (Karahan *et al*., 2011). Several potential risk factors have been reported for subclinical mastitis including; age, lactation stage, parity, udder depth, teat shape, teat size and udder lesions (Hussain *et al*., 2013; Kulkarni and Kaliwal, 2013; Tiwari *et al*., 2013; Qayyum *et al*., 2016). Mastitis incidence is also influenced greatly by management and environmental factors (Steeneveld *et al*., 2008; Ali *et al*., 2014).

Knowledge of mastitis pathogens and their predominance as well as understanding of its risk factors are prerequisites to improve udder health in a herd, region or country. In Pakistan, such information is scarce, even though mastitis is an important cattle disease in the country. The objectives of this research were to evaluate

* Corresponding author: drasghar07@uaar.edu.pk
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the prevalence and risk factors of sub-clinical Mastitis to understand its epidemiology in Pothohar region Rawalpindi district of Punjab Pakistan.

MATERIALS AND METHODS

Study design and setting

A cross-sectional study was conducted from 2017-2018 for a period of one year in Pothohar region of district Rawalpindi, Punjab Province of Pakistan. Pothohar region is in north eastern part of Pakistan, forming northern region of Punjab. Punjab is one of the most livestock populated province in the country (GoP, 2018). All four livestock production systems (Notenbaert *et al*., 2009) exist in the study area. Livestock is one of the major sources of earning and dairy products for the human population of the study area. Commercial as well as subsistent buffalo farms are found here. Dairy farmers keep Nili Ravi, Kundi and cross-bred buffalos in subsistent and commercial farms.

Study population

The study population was lactating buffalos consisting of different breeds such as cross-bred; Kundi and Nili Ravi. Only healthy animals were selected for the evaluation of sub-clinical mastitis kept in commercial and subsistent farms.

Sampling and laboratory diagnosis

A multi-stage cluster sampling technique was used for sampling of healthy animals. At first stage one district (Rawalpindi) was selected randomly out of 36 districts of Punjab Province. In the second stage three Tehsils were randomly selected out of the list of seven tehsils in the district. At third stage of the sampling ten farms were selected randomly in each tehsil (5 commercial and 5 subsistent). At fourth stage six healthy animals were randomly selected from each farm. Total of 196 animals were tested for evaluation of sub-clinical mastitis (Fig. 1).

Before milk sampling quarters were washed by tap-water and dried. Teats were also cleaned with cotton soaked 75% ethyl-alcohol. Then after discarding the first 3 milk streams, 2 ml was collected into a sterile pre-labeled screw cupped test tube. Each selected lactating buffalo was screened for sub-clinical mastitis based on California mastitis test (CMT) (Patterson, 2017). The CMT test was conducted following the previously established procedure (Quinn *et al*., 2004). Two ml of milk from every quarter of udder was taken and gently mixed with 2 ml of CMT reagent in cups of CMT paddle for 15 seconds. Results were concluded based on gel formation such that if there was no gel formation it was categorized as negative and vice versa. Hence, a buffalo was considered positive for mastitis if one or more than one quarters were found CMT positive.

Data variables

The predicting variables studied to identify the potential risk factors included; demography, health, bio-security and management variables (Table I).

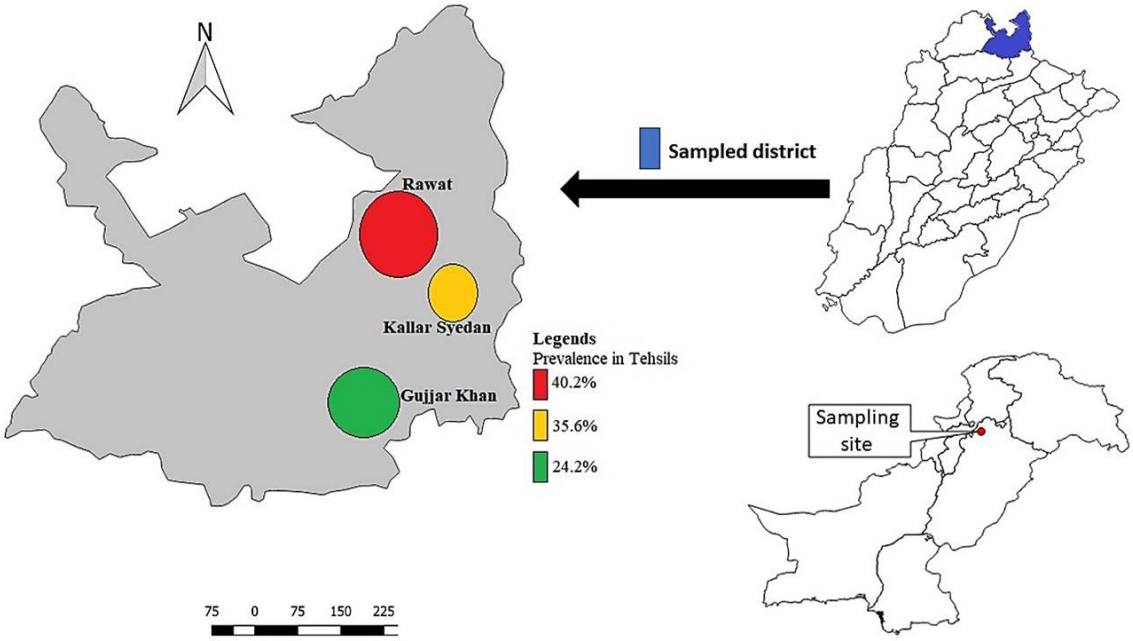

Fig. 1. Geographical representation of sampling area and prevalence at tehsils level in Pothohar region of Pakistan in 2017-18.

Table I.- Predicting variables studied to identify the potential risk factors.

Variables type	Description
Demographic and health variables	Location, age, breeds, body condition score, milk yield, lactation stage, number of lactations, udder shape, teat shape, CMT* test, infected quarter, other disease, milk leakage.
Bio-security measures	Quarantine of new animals, quarantine of infected animals, deworming program, flies controls programs, type of farm, type of shed.
Management variables	Animal movement in shed, source of drinking water, feed type, feed supplementation, udder preparation, dipping used, time of dipping, stimulation of milker, gender of milker, milking techniques, number of milking by milker, used RBSH**, number of specie reared, ratio of buffalo and cattle, animal moved of premises recently, number of people attend animals, animal bedding change, animal manure change, sharing of feed, source of animals for owner, hoof trimming, occurrence of mastitis, doctor availability.

*California Mastitis Test; **Recombinant Bovine Somatotropin Hormone.

The outcome variable was sub-clinical mastitis status on CMT. Data regarding the predicting variables was collected through a pre-designed questionnaire translated into local language. Face to face interviews were conducted by a veterinary officer from the farmers (31.4%), managers (52.2%), and farm owners (16.4%) after signing a consent form by them. This questionnaire contained only close ended questions.

Statistical analysis

Data was validated by cross checking of data in excel sheet with hard copies of questionnaires randomly. Statistical analysis of the data was performed through SPSS version 22.00. A comprehensive list of 43 independent-explanatory variables studied here is given in Table I. "Dummy variable adjustment" method was adopted for missing data management in the predicting variables in the final regression model (Table III) used here (Khan *et al*., 2018). Spatial distribution was performed by using the coordinates data through Arc Map 10.5 using electronic devices at the time of data collection on field. GIS mapping was performed through QGIS version 3.2.2 (QGIS, 2013).

Data normality was assessed using Kolmogorov-Smimov (Sig = <0.001) and Shapiro-Wilk (Sig = <0.001) test. Regression analysis of the data was conducted using univariable and multivariable logistic regression model. Multivariable logistic regression model was developed. Variables having P-value<0.20 were retained in the final logistic model (Table III). Hosmer and Leme show test were applied to check overall model significance and Negalkerke R square for goodness of fit for model (Hosmer *et al*., 2013).

RESULTS

A total of 196 lactating buffaloes were tested by CMT as diagnostic test from commercial and subsistent farms in the pothohar region of Punjab. Amongst the sampled buffaloes 57.7% were Nili Ravi breed; 20.2% Kundi and 22.1% were cross-bred. Most of the buffaloes 106 (54.08%) milk yield was less than 5 liters per day. Source of animal on most of the farms reported by the respondents were mix type such as purchase and by-birth (47.9%) (Table II).

Prevalence of sub-clinical mastitis

Of the 196 screened buffalos through CMT, 132 (67.3%) were found positive for sub-clinical mastitis. Where highest prevalence was found in buffaloes in their early lactation stage (85.2%) (Table II). Buffaloes having 3rd or greater lactation number were found with significantly (P-value=0.01) prevalence of sub-clinical mastitis (88.6%). Prevalence was reported only for those variables which were retained in the final regression model. Variables found significantly associated (P-value<0.05) with sub-clinical mastitis amongst the listed (Table I) included: udder shape, teat shape, udder preparation, dipping status, manure removal frequency, feed sharing and source of animal.

Epidemiology of risk factors

A total of 43 variables were tested in the univariable regression analysis and 23 were found being associated with the occurrence of sub-clinical mastitis or as deterrent factor for subclinical mastitis. Variables with an association with p-value <0.20 were retained in the final model after passing the collinearity screening test. The collinearity between the variables was assessed and those passing the initial univariable screening criteria are listed (Table III). There was a visible considerable collinearity found between several predicting variables, all related to health, management and bio-security.

Amongst the 13 variables (Table III) in final multivariable logistic regression, factors those were significantly associated with sub-clinical mastitis includes; body condition score, lactation stage, no of lactation, udder shape, udder preparation, dipping status, no of people

Table II.- Sub-clinical mastitis prevalence in Pothohar Plateau in buffalo commercial farms in 2018 Pakistan.

Variable name / Description	Positive/ No.	Prevalence	P-Value
Body condition score			
Good	47/64	73.4	0.113
Medium	73/108	67.5	
Poor	12/24	50.0	
Milk yield			
>5	65/106	61.3	0.06
<5	67/90	74.4	
Lactation stage			
Early	87/102	85.2	0.00
Middle	10/35	28.5	
Late	35/59	59.3	
No. of lactation			
First time	63/102	61.8	0.01
2nd time	38/59	64.4	
>3rd time	31/35	88.6	
Udder shape			
Non-pendulous	32/70	45.7	<0.001
Pendulous	22/26	84.6	
Cylinder	37/47	78.7	
Round	41/53	77.3	
Teat shape			
Round	34/63	53.6	0.031
Cylinder	88/121	72.7	
Bowl	7/12	58.4	
Udder preparation			
Yes	43/75	57.3	0.043
No	89/121	73.5	
Dipping status			
Yes	2/8	2.5	0.016
No	130/188	69.1	
No. of people attending animal			
1	102/160	63.7	0.30
2 or >2	30/36	83.3	
Manure removed			
Daily	114/167	68.2	0.005
Once a week	18/29	62.07	
Feed sharing			
No	93/150	62	0.004
Yes	39/46	84.78	
Source of animals			
By Birth	41/71	57.7	0.002
Purchase	29/31	93.5	
Mix	62/94	65.9	
Deworming program			
No deworming	29/35	82.8	0.169
Once a year	56/85	65.8	
Twice a year	21/33	63.6	
Thrice a year	26/43	60.4	

Only those variables are reported here which are having significant association with the occurrence of sub-clinical mastitis. Variables having p-value <0.20 were retained in the final regression model.

Table III.- Multivariable logistic regression model for the risk factors associated with the occurrence of sub-clinical mastitis in commercial buffalo farms, in Pakistan.

Variables / Levels	Standard error	Odds ratio	C.I at 95%	P-value
Body condition score				
Poor	Ref	-	-	-
Medium	0.497	2.765	1.04-7.32	0.041
Good	0.457	2.086	0.85-5.1	0.108
Milk yield				
>5	Ref	-	-	-
<5	0.313	1.837	0.99-3.396	0.052
Lactation stage				
Middle	Ref	-	-	-
Early	0.467	14.500	5.8-36.2	0.000
Late	0.459	3.646	1.48-8.95	0.005
No. of lactation				
First time	Ref	-	-	-
2 or more time	0.556	4.604	1.54-13.68	0.006
Udder shape				
Non-Pendulous	Ref	-	-	-
Pendulous	0.594	6.531	2.03-20.92	0.002
Teat shape				
Round	Ref	-	-	-
Cylinder	0.594	2.559	0.48-13.66	0.272
Udder preparation				
Yes	Ref	-	-	-
No	0.324	1.925	1.02-3.63	0.043
Dipping status				
Yes	Ref	-	-	-
No	0.832	6.724	1.31-34.31	0.022
No. of people attending animal				
1	Ref	-	-	-
2 or >2	0.476	2.843	1.11-7.23	0.028
Manure removed				
Once a week	Ref	-	--	-
Daily	0.494	2.958	1.12-7.78	.028
Feed sharing				
No	Ref	-	-	-
Yes	0.444	3.415	1.43-8.14	0.006
Source of animals				
By Birth	Ref	-	-	-
Purchase	0.763	7.484	1.67-33.37	0.008
Deworming program				
Thrice a year	Ref	-	-	-
Once a year	0.546	3.160	1.08-9.22	0.035

Only those variables are reported here those were having P-value <0.20 in the univariable logistic regression. P-value based on Wald statistics.

attending animals, manure removal frequency, feed sharing, source of animal and de-worming status. Buffaloes at their early lactation stage were at higher risk (OR=14.5; P-value<0.001) of sub-clinical mastitis as compared to late and mid stage animals. Source of animal (purchased) reported by farmers (OR=7.4; p-value=0.008) and teat dipping (No) (OR=6.7; P-value=0.02) in antiseptic was found as a potential risk for sub-clinical mastitis. Buffaloes with pendulous udder shape were 6.5 times significantly (p-value=0.002) at higher risk of infection than buffaloes with non-pendulous udder shape.

DISCUSSION

The aim of the current study was to identify the factors responsible for transmission, spread and increase prevalence of SCM at subsistent and commercial farm level, specific to the local farming system in Pothohar region, Pakistan.

The prevalence of SCM recorded here (67.3) at buffalo level was like the earlier reported by Mekonnen *et al*. (2017) in Ethiopia. Over all prevalence estimated in this study is much higher reported in other studies from Pakistan (Chishty *et al*., 2007; Hameed *et al*., 2012; Mustafa *et al*., 2013; Akhtar and Tanweer, 2016; Qayyum *et al*., 2016; Ali *et al*., 2019). Interestingly in commercial farms, we found high prevalence (77.3%) of SCM than subsistent (22.7%). It could be due to large lack of better management and bio-security practices. Farms where milking was done by whole hand had higher prevalence (72.7%) than machine milking (13.6%). These findings indicate that SCM prevalence have shown increasing trend over the past decade in different parts of Pakistan. It might be by chance, but most probably changes in infection pressure because of increase in animal number, increase in milk yield and other genetic variations in breeds over the time. The increase in prevalence of SCM could also be due to change in climatic parameters in the study area over the last few years. As reported previously by Khan *et al*. (2015) from Pakistan, suggesting that mastitis incidence in influence by season. Day temperature and humidity has been reported influencing mastitis infection in lactating cattle's (Lescourret *et al*., 1995; Steeneveld *et al*., 2008; Breen *et al*., 2009). The higher prevalence of SCM in our study in commercial farms could be due to the unsatisfactory management, and bio-security practices.

Epidemiology of risk factors for SCM

The results indicated that certain predicting variables are associated with SCM in lactating buffaloes of Pothohar region of Pakistan. Broadly certain health, management and bio-security practices contributed in high SCM prevalence in the study area (Table III).

Health related risk factors

Amongst the health-related variables, the potential risk factors found here included; body condition score, milk yield, lactation stage, no of lactations, udder shape (Table III). Buffaloes with early lactation stage were found at higher risk (OR=14.5; CI=5.8-36.2) of SCM; have also previously reported significantly associated with mastitis (Breen *et al*., 2009; Ramírez *et al*., 2014). It might be due to sensitiveness of teat muscles to external pressure when hand milking practices are adopted. As also evident in our results that higher prevalence was found in commercial farms where hand milking practice reported. Lactating buffaloes with 2nd or greater lactation number were 4.6 times at higher risk of SCM than buffaloes with first time. This might be due the increase in exposure to milking process (especially hand milking), also creating an increase probability of exposure to infectious agents. This is also reported by Neave *et al*. (1969). Body condition score (medium, good) and milk yield (>5 liters) were also found as risk for SCM. These are also reported in previous studies as potential risk factors; briefly related to udder shape and teat size (Hussain *et al*., 2013; Hiitiö *et al*., 2017).

Managemental risk factors

Several management predicting variables were screened in this study (Table III). We observed that managemental practices adopted in the study area were unsatisfactory. Where high production herds are usually managed skillfully, and systematic breeding is done (Taponen *et al*., 2017). Whereas, early diagnosis of mastitis is considered as better control and treatment of mastitis in developed World. But here the farmers, managers and owners were unaware of diagnostic procedures and lacks facilities were reported. Udder preparation and dipping was mostly not performed at most farms; found at higher risk of SCM. These results are in line with the findings of Ramírez *et al*. (2014). More people attending the lactating buffaloes were also found as potential risk factor (Ramírez, 2013). Manure removal frequency was a potential risk factor for transmission of SCM here. These factors have been identified in previous studies in different regions globally (Hameed *et al*., 2012; Spoor *et al*., 2013; Rainard *et al*., 2018).

Bio-security Risk factors

In most of the develop countries maintaining a closed herd is the choice of farming. Whenever animals are necessary for lactating herd expansion or replacement, several precautionary measures are taken (Ramirez *et*

al., 2014). Such as; quarantine of newly arrived animals, timely de-worming of animals, which is not practiced in Pakistan due to lack of awareness in amongst the farmers. It's a prime importance of bio-security measures to avoid introduction of pathogens in herd. Due to lack of these measure feed sharing, de-worming, and source of animals were found potential risk factors (Table III) in the study area mostly in commercial farms. These findings are in line with previous studies (Zadoks *et al*., 2011; Hameed *et al*., 2012; Harrison *et al*., 2013; Spoor *et al*., 2013).

Limitations of the study

There were incomplete dairy farms database/ registration in Pakistan and farms were selected based on list provided by private companies for medicine and feed. This might have caused selection biasness in our study by missing other farms not listed in the list provided by a private source. The farms sampled here were not uniform in terms of the number of animals in it. Therefore, the management, bio-security measures, exposure rate, transmission possibility and spread of infection could be different in each farm.

CONCLUSION

Here we identified several risk factors being reported risk factors in previous studies. Showing that these risk factors are mainly responsible for elevated risk of sub-clinical mastitis. Controlling these factors could potentially reduce the risk of sub-clinical mastitis in commercial as well as subsistent dairy farms in buffaloes in Pakistan and in other Asian developing countries.

Considering the findings of this study we recommend prioritize attentions needs to be given for control of management and bio-security measures. Proper management and strict bio-security practices together can control and prevent the transmission and spread of sub-clinical mastitis.

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Statement of conflict of interest

The authors declare no conflict of interest relating to this research work.

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